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Abstracts

Oral Presentations:

Poster Sessions:

OP 1

Using Oral Agents in the Management of Diabetes

1

INCREASED MORTALITY IN TYPE 2 DIABETES PATIENTS USING SULPHONYLUREA AND METFORMIN IN COMBINATION

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Aims: In the UKPDS study, patients allocated to combination treatment with sulphonylurea and metformin (SU+MET) had an increase in diabetes-related death as compared to patients on only sulphonylurea (SSU). The present observational study compared cause-specific mortality in type 2 diabetes patients using SU+MET versus those on SSU.
Materials and Methods: All type 2 diabetes patients identified in two Swedish municipalities from 1984 to1994 using SU+MET or SSU as antihyperglycaemic agents were included and followed until Dec 31, 1996, or until death. Fatal events were identified until Dec 31, 1996. 171 SU+MET users and 872 SSU users were included. Odds ratios calculated by Cox regression analysis were adjusted for age, sex, duration of diabetes, municipality, year of inclusion and fasting blood glucose at inclusion.
Results: As shown below, all cause mortality, IHD mortality and stroke mortality were significantly higher in SU+MET users. The odds ratio for mortality from causes other than IHD and stroke did not differ significantly between the different users.
Conclusions: The results support the finding in the UKPDS study suggesting a less favourable outcome in type 2 diabetes patients on SU+MET.

Number of deaths (underlying cause) (N) and deaths per 1000 person years (N/1000) in users of sulphonylurea as single oral antihyperglycaemic agent (SSU) and in patients using sulphonylurea and metformin in combination (SU+MET). Adjusted odds ratios (OR) associated with use of SU+MET compared to users of SSU.

Cause of	SSU (reference)		SU+MET			
mortality	N	N/1000	N	N/1000	Adjusted OR[1]	
All causes	467	86.2	88	91.3	1.77	(1.39 - 2.26)
IHD	259	47.9	51	52.9	1.95	(1.41 - 2.69)
Stroke	97	17.9	23	23.9	2.16	(1.31 - 3.56)
Other causes	175	17.4	27	11.4	1.27	(0.82 - 1.95)

1. Odds ratios adjusted for age, sex, duration of diabetes, municipality, year of inclusion and fasting blood glucose at inclusion.

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ROSIGLITAZONE IS NOT ASSOCIATED WITH HEPATOTOXICITY

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Aims: To evaluate the effect of rosiglitazone (RSG), a potent thiazolidinedione, on hepatic function in patients with type 2 diabetes mellitus. Troglitazone, the first marketed thiazolidinedione, has been associated with elevated liver enzymes, liver damage, and death secondary to liver failure. **Materials and Methods:** Pooled data from 11 double-blind placebo- or active-controlled studies. Overall, 3455 patients have been treated with RSG, alone or in combination with other antidiabetic agents. Patients were permitted to enter clinical trials with ALT or AST values at screening up to 2.5x the upper limit of the reference range (ULRR). Liver function tests (LFTs) were monitored every 4 weeks for the first 3 months, every 6 weeks for the next 3 months, and quarterly thereafter. Patients completing double-blind studies were eligible to receive treatment with RSG in long-term open-label extension studies. More than 4500 patients were treated with RSG in double-blind and open-label studies: over 2700 for at least 6 months and over 1000 for at least 12 months. **Results:** The proportion of RSG-treated patients with on-therapy ALT >3x ULRR was similar to the proportions of patients treated with placebo or active comparators. Of the 6 RSG-treated patients with on-therapy ALT >3x ULRR, 4 had normal LFTs at baseline. In all 6 patients, ALT returned to normal on continued RSG therapy, except in 1 whose ALT was >3x ULRR prior to RSG treatment but showed no worsening during the study. During open-label treatment, 7 additional patients developed ALT >3x ULRR: 4 improved or normalized on continued RSG therapy, 2 have no follow-up data available, and 1 had ALT >3x ULRR prior to RSG treatment with no worsening during the study. Overall, while 5 patients with elevated ALT values at baseline had increases to >3x ULRR, over 250 other patients with baseline ALT elevations did not have ALT >3x ULRR on RSG therapy. **Conclusions:** Data from more than 4500 patients indicate that RSG is not hepatotoxic.

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INCREASED MORBIDITY AND MORTALITY ASSOCIATED WITH DISCONTINUATION OF ORAL ANTIDIABETIC THERAPIES

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Aims: To evaluate the rate of discontinuation of three oral antidiabetic therapeutic classes (sulphonylureas, alpha-glucosidase inhibitors, and biguanides) and the difference in morbidity and mortality between those continuing vs. those discontinuing therapy. **Materials and Methods:** An inception cohort of 13,309 patients with diabetes mellitus who were over the age of 45 years and who received their initial oral antidiabetic prescriptions for acarbose, metformin, or a sulphonylurea were included in a retrospective analysis of automated primary care data covering 1.9 million patients with visits between 1991 and August 1997. The primary care database represented 653 physicians associated with 145 general practices in the United Kingdom. Patients with juvenile-onset and gestational diabetes were excluded from the analysis. Discontinuers were defined as those patients who failed to refill their oral antidiabetic prescription during the two follow-up periods (6 and 12 months). A survival analysis was performed based on the initial oral anti-diabetic medication class. **Results:** Acarbose users demonstrated the most rapid rate of discontinuation, with only 50% [95% CI 46.4-53.3] of users still on medication after 12 months compared to 73% [95% CI 72.1-74.7] for metformin and 84% [95% CI 83.7-85.3] for the sulphonylureas. Discontinuers were 1.9 times as likely to require emergency hospitalization than continuers ($p<0.05$). All-cause mortality rates among discontinuers were 2.9-fold higher than among those remaining on their medication ($p<0.05$). **Conclusions:** Patients who discontinue oral antidiabetic therapy suffer higher rates of morbidity and mortality. These results underscore the importance of compliance in the treatment of diabetes.

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CONTRA-INDICATIONS TO METFORMIN THERAPY IN PATIENTS WITH DIABETES: A POPULATION-BASED STUDY

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The UKPDS has shown the benefits of metformin (MF) beyond all doubt, yet treatment with MF is associated with the risk of lactic acidosis. **Aims:** To determine population data on the current use of MF and the presence of contra-indications to MF in people with type 2 diabetes. **Methods:** Using the DARTS/MEMO database of all diabetic patients in Tayside (population 349303; 7885 DM, prevalence 2.26%) we investigated the presence of contra-indications to MF in all diabetic patients taking MF in 1993-1995.The contra-indications of acute myocardial infarction (AMI), renal impairment (creatinine>150: RF) and heart failure (CCF) were identified by DARTS, the regional biochemistry database, the hospital admissions database and MEMO prescribing database. **Results:** A total of 1847 (26.2% of DM2) were taking metformin. Of these, 65 (3.5%) were admitted with AMI (71 episodes); 77 (4.1%) were admitted with CCF (114 episodes), 388 (21%) received MF and drugs for CCF concurrently; and 87 (4.7%) had established RF. Contra-indications to MF use rarely resulted in discontinuation of MF therapy; for example only 20% and 18% stopped MF immediately after admission for AMI and CCF respectively. There were no episodes of lactic acidosis.
Conclusions: This population-based study shows that at least 25% of patients who currently receive MF have absolute contraindications to its use. In view of the proven benefits of MF treatment, a critical review of its safety profile and absolute contra-indications is warranted.

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REPAGLINIDE AMPLIFIES INSULIN SECRETORY BURST MASS WITHOUT AFFECTING BURST FREQUENCY.

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Insulin is released in distinct secretory bursts at a frequency of one pulse per 5-15 minutes. Repaglinide is a prandial glucose regulator for treatment of type 2 diabetes mellitus. To examine the actions of repaglinide on insulin release, the insulin secretory pattern was investigated following a single low dose of the agent. Eight healthy male subjects, age (mean±SEM) 25.3±0.7years, BMI 22.1±0.4kg/m^2 were examined in a single dose, double blind, placebo controlled cross-over design. After a ten hour overnight fast, blood sampling was initiated and continued every minute for 120 minutes. After 40 minutes a single dose (0.5mg) of repaglinide or placebo was given. Plasma glucose was allowed to decline only 0.3mM below baseline and maintained by variable glucose infusion. Serum samples were analysed in duplicate for insulin and the concentration time series were subjected to deconvolution analyses and the regularity statistic approximate entropy (ApEn). Mean insulin concentration was increased during repaglinide treatment (basal *vs.* stimulated period, p-values are placebo *vs.* repaglinide) (25.1±3.6pM *vs.* 33.5±4.1pM, p<0.001). Secretory burst mass was increased (15.8±2.2pM/pulse *vs.* 19.6±2.8pM/pulse, p=0.016) and a similar increase was seen in pulse amplitude (6.1±0.9pM/min *vs.* 7.7±1.2pM/min p=0.008). A non-significant increase in basal insulin secretion was observed (2.5±0.3pM/min *vs.* 3.2±0.4pM/min, p=0.062), while there was no changes in interpulse interval (6.8±1.0 *vs.* 5.4±0.4min/pulse, p=0.38). ApEn increased significantly after repaglinide administration (0.623±0.045 *vs.* 0.670±0.034, p=0.04) suggesting less regular oscillatory insulin secretion. In conclusion, a single dose of repaglinide amplifies insulin sectretory burst mass (and basal secretion) with no effect on burst frequency. This effect may be beneficial in Type 2 diabetes characterised by impaired pulsatile insulin secretion.

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REPAGLINIDE TREATMENT IS ASSOCIATED WITH SIGNIFICANTLY LESS SEVERE HYPOGLYCAEMIC EVENTS COMPARED TO SULPHONYLUREA

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Aim. As severe hypoglycaemia (SH) is a relatively rare, but potentially dangerous side effect to sulphonylurea (SU) treatment of Type 2 diabetes, the aim was to investigate if this risk could be reduced when using repaglinide (Re), belonging to a new class of compounds (Carbamoylmethylbenzoic acid derivatives), with a faster onset of action and shorter duration of action (T½ approx. 1 hour) than conventional B-cell stimulating agents. **Material and Methods.** Re was compared to the SU: Glipizide, Glibenclamide, or Gliclazide in one year double blind studies, using identical study protocols. Re was dosed at the 3 major meals in order to improve meal related insulin secretion and thereby decrease post prandial glucose levels. SU was dosed according to labelling and placebo at lunch time, so the dosing pattern appeared to be equal for Re and SU. A total of 761 patients were treated with Re and 367 patients were treated with SU. Hypoglycaemic episodes were recorded together with blood glucose determinations, performed by the patients when symptoms occurred. Blood glucose levels < 2.5 mmol/l, together with symptoms were considered as severe hypoglycaemic events. **Results.** The level of metabolic control was not significantly different between the two groups during the treatment period (HbA_{1c}: 7.1%-7.5%). The absolute rate of SH was 1.31% in the Re group, and 3.27% in the SU group (p< 0.03), with no differences between the 3 SU used. The overall pattern of hypoglycaemia differed: 7 % of the events on Re was below 2.5 mmol/l, 88% between 2.5 and 4.4 mmol/l, and 5% > 4.4 mmol/l, compared to SU where 20% were below 2.5 mmol/l, 78% between 2.5 and 4.4 mmol/l, and 2% > 4.4 mmol/l. **Conclusion.** Re caused significantly less severe hypoglycaemic episodes in comparison to SU . This may be related to the rapid onset and short duration of Re, and because Re is only taken before the main meals, acting as a regulator of the prandial blood glucose excursions which are the highest levels during the day in Type 2 diabetes patients.

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NATEGLINIDE IMPROVES PRANDIAL GLUCOSE EXCURSIONS BY RESTORING EARLY INSULIN SECRETION IN PRE-DIABETIC MONKEYS.

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Nateglinide (NAT, an amino acid derivative) and repaglinide (REP, a meglitinide analog) are new oral agents suggested to offer advantage over sulfonylureas (SUs) due to their rapid/transient insulinotropic actions. However, in rodents the effects of NAT are more rapid and less prolonged than those of REP. **Aims:** to determine if kinetic differences seen in rodents also occur in primates and to explore the impact of insulin kinetics on prandial glucose control. **Materials and Methods:** Vehicle (CMC, 1ml/kg) or equipotent doses of NAT (20mg/kg) or REP (0.1mg/kg) were given orally to IGT Cynomolgus monkeys. Effects on insulin (IRI) and glucose were compared in in the fasted state and when compounds were given 10 min before a liquid meal (time 0). To quantify prandial glucose and IRI excursions, incremental AUCs (0-210min) were calculated. **Results:** NAT and REP had strikingly different kinetics in IGT monkeys. Peak NAT levels (25.9±3.3 μg/ml) occurred at 35 ± 9 min, maximum Δ IRI (+81±23 μU/ml) occurred at 27±8 min post-NAT and IRI returned to baseline within 60 min. In contrast, peak REP levels (50±10 ng/ml) occurred at 56±19 min and maximum Δ IRI (+83±53 μU/ml) occurred at 70±18 min post-REP. Similar glucose nadirs followed dosing with NAT and REP (Δ=-26±1 and -21±2 mg/dl, respectively) but euglycemia was restored by 180 min post-NAT while hypoglycemia and hyperinsulinemia were seen for ≥210 min post-REP. When given 10 min before a meal, NAT but not REP increased IRI pre-prandially (Δ= +52±18 *vs* -1±8 μU/ml), mimicking cephalic insulin release and NAT was more effective than REP to reduce glucose excursions (-78±9% *vs* -53±11%). NAT decreased IRI excursions (-57±16%, p<0.02 *vs* CMC, p<0.05 *vs* REP) whereas REP increased IRI excursions >2-fold *vs* CMC. **Conclusions:** In primates as in rodents, NAT had more rapid and transient kinetics than REP and NAT restored early insulin release. The unique properties of NAT confer significant therapeutic advantages over SUs or REP, namely, better prandial glucose control with no hyperinsulinemia and less hypoglycemic potential.

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ROSIGLITAZONE ONCE OR TWICE DAILY IMPROVES GLYCEMIC CONTROL IN PATIENTS WITH TYPE 2 DIABETES

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Aims: To investigate the efficacy of rosiglitazone (RSG), a potent thiazolidinedione, as monotherapy for patients with type 2 diabetes. **Materials and Methods:** Following a 4-wk placebo (PBO) run-in period, 959 patients were randomized to receive PBO or RSG 4mg/d or 8mg/d given once daily (od) or divided (bd). Patients previously had been treated by diet alone (25%) or with oral antidiabetic agents (75%). Baseline mean fasting plasma glucose (FPG) and mean C-peptide levels for the study population were 12.6mmol/L and 0.93nmol/L, respectively. **Results:** RSG improved glycemic control in drug-naive and previously treated patients. In all RSG groups, HbA1c and FPG decreased significantly without increases in serum insulin levels. The 4mg/od and 2mg/bd regimens were equivalent with respect to lowering HbA1c. Free fatty acids decreased in all RSG groups except the 4mg/od group. Small changes in total cholesterol/HDL ratios were observed, but were not suggestive of increased cardiovascular risk. The incidence of adverse events (AE) was comparable between the RSG and PBO groups, although the proportion of patients withdrawn due to an AE was higher in the PBO group (10.8%) than in any RSG group (≤6.2%). There was no evidence of hepatotoxicity in any treatment group. **Conclusions:** RSG at total daily doses of 4mg and 8mg, administered once daily or divided, was well tolerated and significantly improved glycemic control in patients with type 2 diabetes.

Treatment Group	Baseline HbA1c (%)*	Δ Baseline*	Difference from PBO
PBO (n=173)	8.9±1.52	0.8±1.10†	–
RSG 4mg/od (n=180)	8.9±1.59	0.0±1.40	-0.8††
RSG 2mg/bd (n=186)	8.9±1.54	-0.1±1.42	-0.9††
RSG 8mg/od (n=181)	8.9±1.52	-0.3±1.24†	-1.1††
RSG 4mg/bd (n=187)	9.0±1.52	-0.7±1.37†	-1.5††

*mean±SD; †P<0.001 vs. baseline; ††P<0.0001 vs. PBO

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Epidemiology of Type 2 Diabetes

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A POPULATION-BASED STUDY OF THE ASSOCIATION OF VITAMIN C LEVELS WITH GLUCOSE INTOLERANCE.

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Low intake of fresh fruit and vegetables has been shown to be associated with the development of Type 2 diabetes. The mechanisms underlying this association have not been fully explored, but an effect mediated through vitamin C intake is biologically plausible. Although studies of vitamin C levels in individuals with established diabetes have been undertaken, there are no studies in individuals with previously undiagnosed diabetes or lesser degrees of glucose intolerance.

Aims: The aim of this study was to quantify the association between glucose tolerance and plasma vitamin C levels.

Materials and Methods: A population-based study of diet and chronic disease in which HbA_{1C} and plasma vitamin C concentrations were measured in 2898 men and 3560 women aged 45-74 years.

Results: Mean plasma vitamin C was significantly higher in individuals without glucose intolerance compared to those with self-reported diabetes or prevalent undiagnosed glucose intolerance ($HbA_{1C} \geq 7\%$). An inverse gradient of mean plasma vitamin C was found in both sexes across quintiles of HbA_{1C} distribution below 7%. The odds ratio (95% confidence interval) of having prevalent undiagnosed glucose intolerance per 20 µmol/l (one standard deviation) increase in plasma vitamin C was 0.64 (0.48 - 0.84), adjusted for sex, age, BMI, any dietary supplement taking, vegetarian diet and smoking history. The unadjusted change in HbA_{1C} per 20 µmol/l increase in vitamin C, estimated by linear regression, was -0.12 (-0.14 to -0.09) in men and -0.09 (-0.11 to -0.07) in women. After adjusting for possible confounders these values were -0.09 (-0.12 to -0.06) in men and -0.07 (-0.09 to -0.05) in women. A decrease of 0.1% in the mean HbA_{1C} of this population, would result in a decrease of 14% and 20%, respectively, in the prevalence of undiagnosed glucose intolerance in men and women.

Conclusions: This study demonstrates a strong inverse association between plasma vitamin C and HbA_{1C}. Dietary measures to increase plasma vitamin C may be an important public health strategy for reducing the prevalence of diabetes.

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PROINSULIN, A RISK FACTOR FOR CARDIOVASCULAR DISEASE.

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The extent to which proinsulin may contribute to the association between insulin and cardiovascular disease (CVD) is largely unknown.

Aim: To identify the longitudinal relationship between proinsulin and CVD in a cohort of 50-year-old men (n=874) investigated 1970 - 73 and subsequently followed for up to 26.7 years (mean 22.6 years) from baseline.

Materials and Methods: Fasting concentrations of specific insulin, intact and 32-33 split proinsulin were measured in plasma samples, stored frozen in liquid nitrogen since baseline, by a specific two-site immunometric assay. The diagnosis of incident disease was collected from the official in-patient and causes-of-death registers. Results, adjusted for age at entry, from the Cox proportional hazards regression models using standardised variables, given as hazard ratios (HR) and 95% confidence intervals (CI).

Results: Intact proinsulin (HR=1.69, CI=1.41-2.01) was the strongest predictor for death of CVD, followed by 32-33 split proinsulin (HR=1.44, CI=1.21-1.72), immunoreactive (IRI) (HR=1.44, CI=1.21-1.73) and specific insulin (HR=1.32, CI=1.11-1.58). Intact proinsulin was independent of known confounders in the multivariate analysis (BP, BMI, smoking, LDL/HDL cholesterol ratio, TG and fasting glucose) (HR=1.48, CI=1.20-1.83) while IRI was not (HR=1.12, CI= 0.90-1.40). Intact proinsulin (HR=1.63, CI=1.43-1.85) was the strongest predictor for CVD-morbidity, followed by 32-33 split proinsulin (HR=1.42, CI=1.25-1.61), IRI (HR=1.25, CI=1.09-1.42) and specific insulin (HR=1.21, CI=1.07-1.38). Intact proinsulin was independent of known confounders in the multivariate analysis (HR=1.45, CI=1.25-1.69) while IRI was not (HR=0.99, CI= 0.84-1.14).

Conclusions: We conclude that increased proinsulin predicts death and morbidity in cardiovascular disease independent of known risk factors.

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A COMMON POLYMORPHISM OF THE LEPTIN RECEPTOR GENE IS ASSOCIATED WITH THE RISK OF TYPE 2 DIABETES IN HEALTHY MEN

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Aims: Leptin receptors are expressed in the pancreatic β-cells, where they proposedly mediate leptin-induced inhibition of insulin secretion. Heterozygous carriers of a pentanucleotide insertion in the 3'-untranslated region (3'-UTR) of the leptin receptor gene have been described to have lower serum insulin levels than the homozygous carriers of the more common deletion allele. There are no previous prospective epidemiologic studies of the association of leptin receptor gene polymorphisms with the risk of type 2 diabetes in healthy populations. **Materials and methods:** We studied the association between the carrier status for the 3'-UTR insertion allele and the risk of type 2 diabetes in a prospective population-based case-control study in 41 men who developed type 2 diabetes (fB-gluc ≥6.7 mmol/l or 2h-fB-gluc ≥ 10.0 mmol/l or treatment for diabetes) during a 4-year follow-up and 81 controls who were matched for age, measurements of obesity, baseline glucose and insulin levels and eight other predictors of diabetes. Both the cases and the controls came from a cohort of 985 randomly sampled non-diabetic (fB-Gluc <6.0 mmol/l or no treatment) men aged 42-60 years from eastern Finland. **Results:** There were one homozygote and 22 heterozygotes for the 3'-UTR insertion allele among all 122 men. The carrier frequency of this allele was 9.8% among the cases and 23.5% among the controls. In a logistic regression model adjusting for strongest other predictors, the carriers of the insertion allele had a 79% reduced risk of diabetes (odds ratio (OR) 0.21, 95% CI 0.06-0.77, p=0.019), compared with non-carriers. In a model including only the carrier status for the insertion allele, the respective OR was 0.35 (95% CI 0.11-1.12, p=0.076), equalling to risk reduction of 65%. **Conclusions:** This is the first study to suggest an association between a polymorphism of the leptin receptor gene and the risk of diabetes. Our data provide support to the hypothesis that alterations in the leptin signaling system could contribute to the development of type 2 diabetes.

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PHYSICAL ACTIVITY AND MORTALITY ACCORDING TO GLUCOSE TOLERANCE. THE HOORN STUDY

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Aims - Physical activity is independently associated with a lower mortality risk in the general population. It is also known that regular exercise has a beneficial effect on cardiovascular risk factors and glycaemic parameters. Therefore physical activity is also expected to reduce mortality risk in diabetic patients.

Materials and Methods - The Hoorn Study is a population-based cohort of 2466 subjects, aged 50-75 years. Glucose tolerance was defined according to WHO-criteria. Physical activity was assessed by questionnaire. Subjects were grouped into tertiles of physical activity level (min per day). Until October 1997, 223 subjects died. Relative risks for all cause mortality were estmated by Cox regression.

Results - Among subjects with normal glucose tolerance the age- and sex adjusted relative risk (95% CI) for all cause mortality were 0.59 (0.38-0.90) and 0.84 (0.57-1.22) for subjects in the intermediate and the highest activity group, relative to the inactive subjects. In subjects with diabetes these relative risks were 0.78 (0.36-1.69) and 1.00 (0.50-1.99) respectively. Adjustement for smoking and other prevalent chronic disease did not change the estimates, adjustment for putative intermediating variables (waist-hip ratio, fasting insulin, HDL-cholesterol) weakened the observed lower risk in the intermediate groups.

Conclusions - Contrary to expected, and the observations in the non-diabetic subjects, the diabetic patients did not benefit of physical exercise with respect to mortality risk.

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HYPERFIBRINOGENEMIA, METABOLIC SYNDROME AND CORONARY HEART DISEASE IN TYPE 2 DIABETES: A POPULATION-BASED STUDY

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Fibrinogen has been suggested to cluster with several components of the metabolic syndrome, thus increasing its cardiovascular risk. **Aims**: to assess in a large population-based cohort of type 2 diabetes 1) variables associated with fibrinogen; 2) the relationship between fibrinogen, the number of components of the metabolic syndrome and coronary heart disease (CHD). **Materials and Methods**: we examined 1574 patients (80% of a population-based cohort). Components of the metabolic syndrome were hypertension (systolic blood pressure≥160 mmHg and/or diastolic blood pressure≥95 mgHg), dyslipidemia (triglycerides>2.82 mmol/L and/or HDL-cholesterol <1.03 mmol/L), hyperuricemia (uric acid>416 umol/L) and increased albumin excretion rate (AER ≥ 20 ug/min). CHD was defined on the basis of ECG abnormalities (Minnesota codes). **Results**: Fibrinogen increased lineraly with age, smoking and hypertension; an increasing trend with the increasing number of components of the syndrome (only diabetes, one, two, three or more) was evident in both sexes, even after adjustment for age, smoking, diabetes duration, HbA1c, BMI, and CHD: 328.3±10.5, 347.2±7.2, 373.8±7.0 and 375.7±12.1 mg/dl in men ($p<0.001$ for linear trend); 335.9±10.4, 365.5±5.0, 377.5±5.7 and 383.6±9.5 mg/dl in women ($p<0.001$). Prevalence of CHD also increased linearly (from 19% to 33%, $p<0.001$). **Conclusions**: fibrinogen increases linearly with the number of components of the metabolic syndrome, independently on major confounders, suggesting its role in this cluster of risk factors.

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THE EFFECTS OF DEPRIVATION ON DIABETES PREVALENCE, DIABETIC CONTROL AND CARDIOVASCULAR RISK IN SCOTLAND: A POPULATION-BASED STUDY

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There are no population-based United Kingdom data on the link between diabetes and social deprivation. **Aims:** The DARTS diabetes register of Tayside, Scotland, was used to investigate the link between deprivation and i) prevalence of diabetes ii) diabetes control and iii) cardiovascular risk factors **Methods:** The study population comprised all Tayside residents during the study period (Jan 93 – Dec 95) who could be assigned a 'Carstairs' deprivation category (depcat). This is a postcode-based measure based on four census variables. Patients who had type 1 or type 2 diabetes diagnosed prior to January 1993 were identified from DARTS. Diabetic control was determined from HbA1c values; cardiovascular risk factor information was obtained from hospital and primary care records. **Results:** In the population of 366,849, there were 792 patients and 5,474 patients with type 1 and type 2 diabetes respectively. The prevalence of type 2, but not type 1, diabetes varied by deprivation. People in depcat 6 (least affluent) were 1.6 times more likely to have type 2 diabetes than those in depcat 1 (adjusted odds ratio 1.6; 95% C.I. 1.43-1.82; $p < 0.001$). There was no association between deprivation and glycaemic control or blood pressure. As depcat increased there were increasing proportions of 'current' smokers and decreasing proportions of 'never' smokers in type 1 and type 2 diabetes. There was a clear increase in BMI with increasing deprivation in type 2 diabetes; mean BMI for depcat 1 and depcat 6 was 26.2kg/m^2 and 29.5kg/m^2 respectively; $p<0.001$. **Conclusions:** The association between deprivation and increasing prevalence of type 2 diabetes provides yet more evidence for the increased demand for health care resources in more deprived patient groups. A large part of the increase in prevalence is explained by obesity.

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A METHOD FOR ANALYSING AND INTERPRETING APPARENT VARIATIONS IN DIABETES AND ITS COMPLICATIONS

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Aims To investigate whether aggregated data from diverse diabetes information systems (DIS) can be analysed robustly to determine real geographical differences (clusters) in the prevalence of diabetes and diabetes-related amputations (DRA).
Methods Data (1997) were amalgamated from 10 adjoining DIS in North-West England (3 different manufacturers; 7 clinic based, 3 population based). The age-sex adjusted prevalence of diabetes and DRA were calculated for each electoral ward, defined by postcode. After adjusting for social deprivation (Carstairs' quintiles; CQ), ethnicity and hospital, expected prevalence and relative risks were examined using tests for heterogeneity of risk and empirical Bayes methods to smooth for the large component of random variation.
Results 31, 469 people with diabetes and 422 diabetes related amputations were recorded in 230 wards (total population 1,809,021, prevalence diabetes 1.7%). Analysis of RR of diabetes demonstrated: significant unexplained residual heterogeneity ($p<0.0001$); associated with increased deprivation (RR=1.34 for deprived compared to affluent, 95%CI = 1.09, 1.58); a 1.91 fold between hospital difference. Raw data for DRA demonstrated numerous apparent "black-spots" (RR > 1.40); deprivation, hospital effects and random variation explained the majority of these differences.
Conclusion If such data routinely collected during clinical care are analysed using appropriate statistical methods, it may be possible to derive robust measures of geographical variations in the prevalence of diabetes and its complications. These methods could identify areas of effective care and would be suitable for health services (St Vincent) monitoring.

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IMPLICATIONS OF CHOLESTEROL MANAGEMENT GUIDELINES FOR DIABETES: A POPULATION BASED STUDY

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Statins are effective drugs in the primary and secondary prevention of coronary heart disease (CHD). Guidelines exist based upon absolute CHD risk assessed from multiple risk factors and derived from the Framingham risk equation. **Aims:** To determine population based data on the implications of lipid lowering guidelines in type 1 (DM1) and type 2 (DM2) diabetes. **Methods**: Using the DARTS/MEMO database (1997-1998) of cardiovascular disease and risk factors (total cholesterol (TC)/HDL, smoking, age, BP, sex) of all diabetic patients in Tayside (population 364880; DM2 n=7127; DM1 n=1055) Scotland, we applied guidelines based upon the 4S study and the Framingham risk equation (secondary prevention: TC>5.0mmol/L; primary prevention >30% risk of CHD over 10 years). **Results:** In DM1 53 (8.9%) of males (M) and 36 (8.4%) of females (F) had a previous macrovascular event; the corresponding figures for DM2 were 1384M (37.2%) and 1166F (34.3%) respectively.Of those <75 years old, 55.3% M and 76.5%F of DM1, and 58%M and 73%F of DM2 had TC>5.0 mmol/L and were eligible for statin therapy. In the 537M and 423F (<75 years) with DM1 in the primary prevention group, the estimated 10 year risk of CHD was 0-15% in 93%M, 98%F; and, 15-30% in 7%M, 2%F. No subjects with DM1 (primary prevention) had >30% risk of CHD over 10 years. In the 1893 M and 1575 F (<75 years) with DM2 in the primary prevention group, the estimated 10 year risk of CHD was 0-15% in 27%M, 32%F; 15-30% in 49%M, 55%F; and >30% in 24%M, 13%F respectively. **Conclusions:** These population data demonstrate that current guidelines recommend lipid lowering therapy for 8.6%M and 7.6%F with DM1, and 36%M and 31%F with DM2, aged <75 years. Unmet need for secondary prevention is >70% in females with both DM1 and DM2. We believe the Framingham risk equation significantly underestimates risk in DM1.

OP 3

Experimental Immunology I

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STREPTOCOCCAL WALL COMPONENT (OK432) RESTORES SENSITIVITY OF NOD THYMOCYTES TO APOPTOSIS

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OK432, a streptococcal wall component, prevents type 1 diabetes in the NOD mouse and the BB rat. Transfer and cotransfer experiments have revealed the presence of suppressor cells, but elimination of suppressor cells by cyclophosphamide could not break the protection, suggesting elimination of effector cells as mechanism of protection. **Aim** : The aim of this study was to investigate whether treatment of NOD mice with OK432 resulted in enhanced sensitivity of NOD lymphocytes to apoptosis signals. **Materials and methods** : Male NOD mice were treated with OK432 (0.1mg/kg) administered intraperitoneally once a week or with the treatment vehicle (PBS). PBS injected male C57Bl/6 mice served as controls. Apoptosis was measured by the TUNEL method in thymocytes 16h after cyclophosphamide (70mg/kg) or 24h after dexamethasone (0.2mg/kg) and in splenocytes 16h after cyclophosphamide injection. **Results** : Apoptosis after dexamethasone in thymocytes was as expected lower in NOD (45±3%) compared to C57Bl/6 mice (63±5%, $p<0.005$) but treatment with OK432 clearly enhanced the sensitivity to the apoptosis signal in NOD mice (62±4%, $p<0.005$). After cyclophosphamide again a clear improved apoptosis rate was observed in the OK432 treated mice in thymocytes (18±4% vs 8±1% in control NOD ($p<0.05$) and 22±4% in C57Bl/6), but not in splenocytes (13±1% vs 15±2% in control NOD (NS) and 35±2% in C57Bl/6). In a second part of the study the experiments were repeated in female mice. First we confirmed the previously observed sex difference in apoptosis sensitivity, but also in female mice OK432 enhanced the sensitivity of thymocytes (11±2% vs 6±1% in control NOD ($p<0.05$) after cyclophosphamide and 39±5% vs 26±3% in control NOD ($p<0.05$) after dexamethasone) but not splenocytes (8±1% vs 10±1% in control NOD (NS) after cyclophosphamide). **Conclusion** : These data indicate that the elimination of effector cells by OK432 in NOD mice might be induced through better elimination of these cells centrally in the thymus, by improved apoptosis.

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Diet promotes β-cell loss by apoptosis in prediabetic NOD mice

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Abstract
Diet as an environmental factor influences age of onset in models of spontaneous insulin dependent diabetes mellitus. We reported recently that a protein - rich diet accelerated diabetes incidence in NOD mice. In the present study, we investigated the effect of diet on β-cells and glucose metabolism of NOD mice before diabetes onset. Three different diets were maintained from 4 weeks on: LF (12% fat, 21% protein, 68% carbohydrates), HF (39% fat, 17% protein, 43% carbohydrate), and HFHP (43% fat, 38% protein, 19% carbohydrates) diet. The cumulative incidence of diabetes was 92% for HFHP, 80% for HF (n.s.) and 65% ($p<0.01$) for the LF cohort. At 20 wk of age insulin secretion of the isolated pancreas was doubled for the HF diet and 4.4 times higher for the HFHP fed mice compared with the LF group. Feeding HF and HFHP reduced total glucose utilisation during continuous insulin infusion (1 mU/kg) by 34% ($p<0.05$). HFHP, but not HF diet elevated endogeneous glucose production by 48% ($p<0.05$) compared with the LF group. β-cell mass estimated by imaging analysis was initially high in young HFHP fed mice aged 10 wk, but declined rapidly thereafter (HFHP 1.6 ± 0.2 ($p<0.05$ vs. LF), HF 2.4 ± 0.4 (n.s. vs. LF), and LF 2.1 ± 0.5 mg at 30 wk). Reduction of β-cell mass was associated with HF 14% ($p<0.05$ vs. LF) and HFHP 82% ($p<0.01$ vs. LF) more apoptotic β-cells at 30 wk. Depending on age, 1.2 - 3.1 of 1000 β-cells were in a stage of proliferation without significant differences among the dietary groups. In conclusion, HFHP diet was associated with impaired glucose metabolism and high insulin release followed by enhanced diabetes incidence. Diabetes was promoted by increased rate of cell death over β-cell neogenesis.

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DECREASED INTESTINE PERMEABILITY, ALTERED VILLUS ARCHITECTURE AND BRUSH BORDER ENZYMES IN SMALL INTESTINE OF NOD MICE

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Aims: The involvement of gut mucosal immune system has been reported in ethiopathogenesis of IDDM. Gut development and maturation may play a critical role in balancing the gut mucosal immune system. We have previously reported the impairment of gut mucosal immune system in NOD mice. In this study we investigated intestine permeability, villus architecture and activities of brush border enzymes within the gut mucosa of NOD compared to BALB/cJ female mice. **Materials & Methods:** Using gas chromatography assay, the intestine permeability of 8-week-old NOD versus BALB/cJ mice was determined by measuring the lactulose/rhamnose ratio in urine. Jejunal activities of lactase, sucrase, dipeptidyl peptidase IV (DPP IV), glucoamylase, γ-glutamyltransferase and alkaline phosphatase were assessed. Villus architecture was examined by scanning electron microscopy. **Results:** Significantly decreased intestine permeability, measured as urine lactulose/rhamnose ratio (0.039±0.004 vs. 0.332±0.131 SEM; $p<0.05$) was found in NOD compared to BALB/cJ mice. No significant changes were observed in jejunal activities of DPP IV, glucoamylase and γ-glutamyltransferase. Interestingly, immature character of jejunum in NOD mice was documented by significantly increased lactase (9.42±1.38 vs. 6.14±0.53; $p<0.05$) and decreased sucrase (12.06±0.52 vs. 18.95±1.48; $p<0.001$) activities in nkat/mg compared to BALB/cJ mice. In addition, alkaline phosphatase values, in nkat/mg, were substantially increased in NOD mice (12.99±1.02 vs. 8.52±0.29; $p<0.01$). Finally, scanning electron microscopy revealed substantially smoother villus architecture in jejunum of BALB/cJ compared to NOD female mice. **Conclusion:** Thus, our results showed a decreased intestine permeability, altered villus architecture, and immature-activated enzyme profile of the small intestine of pre-diabetic NOD compared to BALB/cJ mice. We suggest that decreased intestinal permeability in NOD mice could lead to failure in proper, early antigenic stimulation of gut mucosal immune system. The impairment of gut mucosa in NOD mice may serve as a gateway for autoimmunity, e.g. by failing to shape the proper mucosal immune response to outer environmental stimuli.

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AUTOREACTIVE T_H-CELLS OF BB RATS DO NOT ATTACK RIN-CELLS

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Aim: T-lymphocytes from Type-1 diabetic patients and NOD mice bind to xenogeneic rat insulinoma (RIN) cells. Therefore, it was concluded that RIN-cells express diabetes-relevant antigens and that they are suitable targets for recognizing autoimmune T-cells in diabetic and prediabetic patients. We, therefore, investigated whether specific cell subsets from BB rats are cytotoxic to RIN5AH-cells. Additionally, we determined the expression of MHC- and adhesion molecules on RIN-cells because cytotoxicity is decisively influenced by these surface antigens. **Materials and Methods:** Cytolytic reactivity of effector cells was quantified by the release of ^{51}Cr from damaged RIN-cells in comparison to syngeneic islets. Whole splenic MNC's, peritoneal macrophages, positively separated T_H-cells and NK-cells from young normoglycaemic BB- and control LEW.1W rats served as effector cells. Antigen expression on RIN- and islet cells was measured by FACS analysis using the monoclonal antibodies OX18 (MHC class I), OX6 (MHC class II) and 1A29 (ICAM-1). **Results:** In the cytotoxicity assay, MNC's from 23.3% of BB rats destroyed RIN-cells. Cytolytic reactive MNC's directed to islets were detectable in 65.6% of BB rats. RIN-cell lysis was caused by NK-cells in 66.7% and by T_H-cells in 11.1% of BB rats. But also NK-cells from 58.3% of LEW.1W rats showed cytolytic reactivity to RIN-cells. Damage of syngeneic islets was realized by T_H-cells in 77.8% of BB rats. Additionally, in 25.0% and 16.5% of BB rats macrophages and NK-cells, respectively, showed islet-directed reactivity. MHC class I and class II antigens were expressed on 94.0±0.6% and 6.8±1.2% of RIN-cells. Their expression was significantly lower on islet cells (class I: 65.9±3.4%, class II: 2.4±0.6%, $p\leq0.01$). ICAM-1 was present on 92.5±0.8% of RIN- and 4.2±0.7% of islet cells ($p\leq0.01$). **Conclusions:** The results suggest, that autoimmune islet damage in BB rats is mainly mediated by T_H-cells, while further signals seem to be necessary for RIN-cell lysis by this cell subset. Therefore, for recognition of autoimmune T-cells RIN-cells are not suitable as target in the cytotoxicity assay. The observed NK-cell mediated lysis of RIN-cells could be due to the significantly increased expression of ICAM-1 molecules which may facilitate the interaction between NK- and RIN-cells.

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RESCUE OF REMAINING β-CELLS AT ONSET OF TYPE 1 DIABETES IN THE BB RAT.

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β-cell rest can be induced by the use of potassium channel openers (PCOs) which via interaction with the ATP-dependent potassium channels on these cells block the glucose mediated β-cell depolarisation and inhibits insulin secretion. At diabetes debut - defined as a blood glucose level above 8.5 mM - BB rats were maintained on intensive insulin therapy for eight days by subcutaneous implantation of an insulin tablet persistently delivering 1 U/day. Furthermore, the animals received either 100 mg/kg/day of diazoxide - a well-known PCO, or 0.2 ml of vehicle by oral gavage. On day nine the insulin tablet was explanted and the rats were fasted overnight. During the fast, a minimum dose of long-acting zinc protracted bovine insulin was administered sc. to avoid severe ketoacidosis. The rats underwent a stimulated insulin secretion test by intravenous administration in the tail vein of the secretatogue arginine (0.5 g/kg). If the blood glucose level was below 10 mM at the time point t=0, 1 g/kg of glucose was injected 2 min prior to the arginine administration to potentate the insulin secretory effect. Blood samples for C-peptide concentration determinations were taken at the time point t=1, 3, 5, 10, and 15 min after arginine administration. The rats were sacrificed and three parts of the pancreas were excised for immunohistochemical evaluation. 37.5% of the diazoxide treated rats (3/8) retained a normal C-peptide response, whereas none of vehicle treated rats (0/8) exhibited a C-peptide response. Immunohistochemical staining for insulin and glucagon showed that the C-peptide responding rats all had insulin containing pancreatic islets with near-normal peripheral distribution of glucagon-containing α-cells, while islets from non-responding rats displayed heavy infiltration reaching end-stage with α-cells occupying most of the islet area.

In conclusion, target cell rest by use of the PCO diazoxide may be a prominent contributor to rescue of β-cells resulting in a dampening or even prevention of manifest autoimmune diabetes when initiated at early states of the disease development.

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DOSE DEPENDENT EFFECT OF PROPHYLACTIC INSULIN: HIGH DOSES CRITICAL FOR PREVENTION OF DIABETES.

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Prophylactic subcutaneous insulin administration can prevent the development of diabetes in NOD mice and BB rats and is currently being studied in first degree relatives with high risk of developing type 1 diabetes. To explore the potential of this therapy, we investigated the influence of insulin dose and treatment regime.

Female NOD mice were treated from 12 weeks of age with daily subcutaneous insulin injections of 7.2 U/kg, 0.3 U/kg, or buffer. At 47 weeks of age only 38% (8/21) of NOD mice treated with the high dose had developed diabetes. In contrast, 87% (20/23) of the mice treated with the low dose had developed diabetes ($p<0.05$) similar to the 75% (18/23) of the buffer treated.

Insulin must be given frequently to be protective. Thus, using even higher doses (21.6 U/kg) and starting treatment early (5 weeks of age), 37% (13/35) of mice treated five times a week developed diabetes compared to 80% (16/20) of mice treated twice a week with insulin or with buffer alone ($p<0.05$).

In conclusion, these studies demonstrate the critical importance of dose level and treatment regime for preventing diabetes in the NOD mouse. Dose levels similar to what is currently being used in human trials (0.3 U/kg) do not appear to prevent disease in this animal model.

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A novel poly (ADP-ribose) synthetase inhibitor, 5-iodo-6-amino-1,2-benzopyrone, protects mice from multiple-low-dose-streptozotocin induced diabetes.

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The activation of poly (ADP-ribose) synthetase (PARS) in islet ß-cells is thought to critically reduce cellular NAD levels resulting in an inhibition of cell functions and ultimately cell necrosis. Multiple-low-dose-streptozotocin (MLDS) treatment of mice induces insulitis and progressive hyperglycemia. The aim of this study was to determine if a novel, potent, non-toxic and effective PARS inhibitor, 5-iodo-6-amino-1,2-benzopyrone (INH_2BP), could protect against MLDS induced diabetes in BALBc mice. Male mice were treated with INH_2BP (20 or 60mg/kg/day by gavage) for 1 week prior to the first streptozotocin (stz) injection and throughout the study. Diabetes was induced by IP injection of stz (40mg/kg body weight) or vehicle (citrate buffer) on five consecutive days, blood glucose was monitored over a 21-day period after the first stz injection. Pancreas samples were taken on day 21 and total insulin extracted and expressed as ng insulin/mg protein. The stz treated mice developed hyperglycemia over the 21 days, blood glucose levels of 90.8±2.9 and 232±1mg/dl ($p<0.05$, n=12) on day 1 and 21 respectively, with 83% of the mice diabetic (blood glucose > 200mg/dl) on day 21. The lower dose of INH_2BP (20mg/kg/day) had no effect on diabetes incidence or blood glucose levels but mice treated with 60mg/kg/day INH_2BP had a mean blood glucose of 163.4±18mg/dl on day 21 which though higher than control mice (95.5±3.4mg/dl, $p<0.05$) was significantly lower compared to mice treated with stz alone ($p<0.05$, n=12), diabetes incidence was also reduced from 83% to 33% ($p<0.05$ vs. stz alone). Stz treatment decreased pancreas insulin content from 86±5.6 to 22.4±3.8 ng insulin/mg protein ($p<0.05$), INH_2BP at both doses, 20 or 60mg/kg/day, prevented this decrease, insulin content 51±12 and 61.1±11.8 ng insulin/mg protein respectively ($p<0.05$ vs. stz alone). $PARS^{-/-}$ mice were also less susceptible to MLDS having a delayed onset and reduced incidence of diabetes compared to $PARS^{+/+}$ mice on day 21, reduced from 87.5% to 50% ($p<0.05$ vs. $PARS^{+/+}$). In conclusion this data suggest that activation of PARS plays a role in the pathogenesis of type I diabetes.

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REGULATION OF TYROSINE PHOSPHATASE IA-2 GENE EXPRESSION IN INS-1 INSULINOMA CELLS

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Aims: The tyrosine phosphatase-like protein IA-2 has been described as major target autoantigen in type 1 diabetes. Since the function and the regulation of IA-2 is still poorly defined, we here investigate the effects of metabolic factors and immunomodulatory cytokines on expression of IA-2 mRNA levels.

Material and Methods: To measure IA-2 mRNA expression we developed a quantitative RT-PCR using internal homologous competitors for ß-actin (to normalise mRNA levels) and IA-2 (to quantify IA-2 mRNA levels). Studies were performed with the partial glucose sensitive INS-1 insulinoma cells. Cells were stimulated for 6h – 6 days with glucose (125-375 mg/dl), tolbutamide (5-10 µg/ml), forskolin (10 µM), TNFα (200-1000 U/ml) IL-1ß (10-100 U/ml), INFγ, and IL-4 (10-500 U/ml).

Results: Glucose affects IA-2 mRNA expression in a time- and concentration-dependent manner. The maximal stimulation (285±68% of control) was achieved by long term exposure to high glucose concentration (6 days, 375md/dl) (n=3, $p<0.05$). The maximal stimulation (451±85%, n=4) was observed after incubation with forskolin (24h, 10µM). Treatment with TNFα (1000 U/ml, 24 h) and IL-1ß (60 U/ml, 24 h) resulted in a 2-3 fold suppression of IA-2 mRNA levels (36±16% and 40±17% of control, n=4, $p<0.001$). In contrast, tolbutamide, IL-4 or INFγ did not show any significant effect.

Conclusions: This study provides the first data on the regulation of IA-2 mRNA expression. Since glucose and forskolin lead to a significant increase in IA-2 mRNA levels, our data suggest that IA-2 may be involved in glucose-induced insulin secretion and that the regulation may be mediated by cAMP. The importance of the inhibitory effect of the diabetogenic cytokines IL-1ß and TNF-α on IA-2 autoantigenicity is under investigation.

OP 4
Nephropathy I

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ANGIOTENSIN CONVERTING ENZYME INHIBITORS MODULATE THE HIGH GLUCOSE EFFECTS ON EXTRACELLULAR MATRIX SYNTHESIS.

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Alterations in extracellular matrix (ECM) metabolism have been involved in the pathogenesis of diabetic nephropathy and angiotensin converting enzyme inhibitors (ACEIs) have been shown to modify ECM protein synthesis in *in vitro* studies. **Aims:** to evaluate the effects of elevated glucose concentrations and of ACEI on the extracellular matrix production by mouse glomerular epithelial cells (GEC). **Materials and Methods:** GECs were incubated in media containing physiological (5mM) and elevated glucose (30mM) concentration for four days. Then, an ACEI (enalaprilat), 0.3 mM, was added to all experimental conditions for 24 hours. Laminin, type IV collagen and fibronectin cell content and extracellular release into the medium were studied using 35-S methionine metabolic labelling and immunoprecipitation. **Results:** laminin was mainly (85%) present in the cell extract while incubation of cells in high glucose determined only an increase of the released glycoprotein fraction ($p<0.01$). Type IV collagen was completely intracellular and the production was not modified by high glucose. Fibronectin was completely released into the medium and its production was not modified by high glucose. Following incubation with ACEI, laminin production was significantly decreased in high glucose cultured cells ($p<0.001$). Presence of ACEI prevented the production of type IV collagen in all experimental conditions. The ACEI determined an increase of fibronectin released exclusively by high glucose cultured cells ($p<0.001$). **Conclusions:** these results indicate that in the presence of high glucose ECM protein production is modified. ACEIs appear to greatly reduce the concentrations of the two principal structural components of the basement membrane, laminin and type IV collagen. The reported improvement of renal function in diabetic patients treated with ACEI may in part be due to a modulatory effect on ECM production.

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CALCIUM RELEASE BY ANGIOTENSIN II AND GROWTH RATE IN FIBROBLASTS FROM TYPE I DIABETIC PATIENTS.

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Aims: Whether an alteration in intracellular free calcium (Ca^{2+}_i) mobilisation after exposure to angiotensin II(AII) is associated to an increased cell proliferation in cells from type I diabetic patients with nephropathy is unknown. We therefore examined Ca^{2+}_i changes after acute exposure to AII and celll proliferation in cultured skin fibroblasts from 10 type I diabetic patients with nephropathy (DN), 9 without DN (matched for age, diabetes duration and metabolic control) and from a group of 7 matched normal controls (C). **Materials and Methods:** Ca^{2+}_i was investigated in monolayers of cultured skin fibroblasts after six passages. Spectrofluorophotometric Ca^{2+}_i measurements were performed using Fura-2. Cell proliferation was determined by cell count. **Results:** (mean±SEM) Basal Ca^{2+}_i in quiescent (24h serum-deprived) fibroblasts was 71±4 nM in type I diabetic patients with DN, 54±3 in those without and 72±3 in C. AII evoked a fast and transient rise in Ca^{2+}_i, which was higher in fibroblasts from type I diabetic patients with DN (240±17 nM; $p < 0.001$) than that observed in cells from patients without DN (145±9) and in C (130±2 nM). The AII-induced changes of Ca^{2+}_i were completely blocked by the specific inhibitor Losartan (100nM). Cell proliferation rates were higher in patients with DN (mean doubling time 3.8±0.8 day ; $p < 0.05$) than in those without (7.8±2) and normal controls (7.9±1.9). In the whole population of diabetic patients, the increase in Ca^{2+}_i was inversely related with mean doubling time ($r = -0.45$; $p<0.001$). **Conclusions:** In conclusion, we have described an association between increased cell proliferation rate and responsiveness of Ca^{2+}_i to AII in fibroblasts from type I diabetic patients with DN. This increased responsiveness, persisting in long-term cultured fibroblasts, suggest that the increased susceptibility to nephropathy resides in an intrinsic (possibly genetic) determined host-cell response to diabetes.

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INHIBITION OF MITOGEN-ACTIVATED PROTEIN KINASE NORMALISES CELL GROWTH IN DIABETIC NEPHROPATHY.

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Faster cell proliferation rate characterises human skin fibroblasts from IDDM patients with nephropathy. **Aim:** To evaluate whether the inhibition of mitogen-activated protein kinase (MAPK), a key point of the intracellular pathway, may allow to control excess proliferation in diabetic nephropathy. **Materials and Methods**: We evaluated the effect of PD 098059, a specific inhibitor of MAPK on the proliferation rate of fibroblasts obtained from 40 patients with IDDM (20 with established diabetic nephropathy and 20 age, sex and duration of IDDM matched patients with normoalbuminuria) and 10 age and sex-matched non-diabetic control individuals. Cell proliferation rate was measured by direct cell count (Cell Coulter) at the 6th coltural passage in presence or absence of PD 098059 (kind gift of Parke-Davis, Milan, Italy). **Results:** Proliferation rate was faster in fibroblasts from patients with (0.175±0.009 10^5cells·day^{-1}, mean±SEM) than without nephropathy (0.110±0.009, p=0.0001) and from non-diabetic individuals (0.094±0.008, p=0.0001). Inhibition of MAPK induced a reduction of proliferation rate that was larger in patients with nephropathy (0.079±0.006 10^5cells·day^{-1}, 55% reduction) when compared to patients with normoalbuminuria (0.068±0.006, 38% reduction) and to non-diabetic controls (0.064±0.006, 32% reduction), so that proliferation rates were similar among the three groups studied under these conditions. **Conclusion:** Inhibition of MAPK normalises cell proliferation rate in fibroblasts from IDDM patients with nephropathy, suggesting the MAPK activation pathways as possible origin of this dysfunction.

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ACTIVITY AND ISOFORM EXPRESSION OF PROTEIN KINASE C IN CULTURED FIBROBLASTS FROM TYPE 1 DIABETIC PATIENTS.

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Aims: Abnormal activation of PKC by hyperglycaemia may underlie some of the long-term complications of diabetes. To verify whether individual susceptibility to diabetic nephropathy (DN) resides in an intrinsic different host-cell response to diabetes, we compared the effect of different glucose levels on the activity and isoform expression of PKC in cultured fibroblasts from 10 type 1 diabetic patients with DN with that in cells of 10 patients without DN. Nine normal subjects of similar age and BMI served as control. **Materials and Methods:** Forearm skin fibroblasts were cultured (6 passages) in either 5mM (NG) or 20 mM (HG) glucose concentrations. PKC activity was measured by *in situ* PKC assay that used a specific peptide PKC substrate in digitonin-permeabilized fibroblasts. PKC isoforms were determined in cytosol and membrane fractions by Western blot analysis. **Results:** (mean±SD) In NG, *in situ* PKC activity was significantly higher in type 1 patients with DN (14301±1280γ-^{33}P cpm/ mg protein/ min ; $p<0.01$) than in those without (8281±809) and normal controls (7341±774). This difference was due to increased levels of PKC α in the membrane fraction of fibroblasts from type 1 patients with DN. No difference was observed in the expression of PKC ε and ζ in fibroblasts in all the groups. Isoform βII was not present in these cells. Incubation in HG induced a significant increase in PKC activity compared to NG only in fibroblasts from patients with DN (from 14301 ± 1280 γ-^{33}P cpm/ mg protein/ min to 19551 ± 1530 ; $p<0.01$), with no significant changes in cells from diabetic patients without DN and normal subjects. This effect of HG was not associated with significant changes in PKC isoform expression. **Conclusions:** In NG, an increased PKC α expression may explain the higher PKC activity in fibroblasts from type I diabetic patients with DN. The further increase induced by HG observed in these patients was not associated to significant changes in PKC isoform expression.

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ANTIBODIES TO KIDNEY'S BASAL MEMBRANE IN PATIENTS WITH DIFFERENT STAGES OF DIABETIC NEPHROPATHY

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Aims: There are some evidences that immune mechanisms may play a role in the pathogenesis of diabetic nephropathy. Therefore, the aim of this study was to investigate the presence of antibodies (AB) to basal membrane of kidney's glomeruli in plasma of patients with IDDM with different stages of nephropathy. **Materials and Methods:** We studied 56 IDDM patients aged 16-50 years (mean age 28.6±1.1 years, data presented as mean±SEM) with the mean duration of diabetes 12.6±1.1 years and 24 age-matched controls. AB to basal membrane were determined using specific radioimmune assay. The results between groups were compared using x^2 and Fisher tests. **Results:** In control group there was no presence of AB to kidney's basal membrane. We found that AB were present in 15 patients with IDDM (26.7%). In the group of IDDM patients with the presence of AB to basal membranes the high concentration of AB was noticed in 6 patients while in 9 people it was moderately increased level of AB. Subsequently, we analyzed the presence of AB in IDDM patients with different stages of nephropathy using Mogensen's staging system. AB were found in 3 patients without nephropathy (21.4%), in 1 patient with nephropathy 1-11 stage (14.3%), in 5 of those with nephropathy 111 stage (29.4%), in 6 subjects with nephropathy IY stage (42.9%), and in none of patients with end-stage renal disease. **Conclusions:** We may conclude that AB to basal membrane of kidney's glomeruli were present in plasma of some patients with IDDM. The number of people which positive reaction to these AB increased with progression of diabetic nephropathy while this reaction was negative in those with end-stage renal failure. We speculate that production of AB to basal membrane of kidney's glomeruli may contribute to progression of nephropathy in patients with IDDM being secondary phenomenon developing in response to primarily basal membrane damage.

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Renal Hypertrophy is Reversible with Longterm Lisinopril Therapy in Children with Type I Diabetes Mellitus

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Introduction: Children with Type I diabetes mellitus develop significant renal hypertrophy during the early years of their disease. If this hypertrophy correlates with renal hyperfiltration it should be reduced by administration of an ACE-inhibitor. However there are no published data in children, including in the phase before micro-albuminuria develops.

Aim of the study: To assess the effect of the ACE-inhibitor lisinopril on reversibility of renal hypertrophy.

Methods: Stable Type I diabetic children, without micro-albuminuria. Renal morphology, studied by ultrasonographic (US) measurement of longitudinal, ventral-dorsal and medial-ventral axis (Ultramark 8 sector-scan 5 or 3 MHz). Comparisons were made against normal values of a control population of 200 children (0-16 y). Values were expressed as percentage of the linear regression line (length + 9.513)+17.66 = mean axis-length/body-length: controls 100±8%.

Study-population: F=14, M=9; age at onset of diabetes, 6.4±3.3y; duration of diabetes at first examination, 5.7±1.7y. Duration of lisinopril therapy, 3.8±0.6y.

Results: No significant difference for insulin dose (0.92±0.28/97±0.35), HgA_{1C} (10.2±2.8/9.8±2.7) or creatinine clearance pre- and post-treatment Statistics = paired T-test, * = $p < 0.03$ ** = $p < 0.001$

US renal axis	Left Kidney before ACEi	Right Kidney before ACEi	Left kidney + ACEi	Right kidney + ACEi
Longitudinal	109±12%	107±6%	99±7%*	98±7%**
Medial-lateral	112±8%	113±8%	101±8%**	101±8%**
Ventral-dorsal	121±12%	122±11%	104±8%**	104±9%**

Conclusion: Renal hypertrophy in children with Type I diabetes is reversed by long term lisinopril therapy. Decreased renal volume is not associated with decreased renal function.

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IMPACT OF ANGIOTENSIN CONVERTING ENZYME INHIBITION ON THE PROGRESSION OF MICROALBUMINURIA IN TYPE 1 DIABETIC PATIENTS: AN AUDIT

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Aims: In untreated type 1 diabetic patients with microalbuminuria (MA) a progression rate of urinary albumin excretion (UAE) of 10-15 %/yr have been reported by several groups including our center. Intervention studies have demonstrated a beneficial effect of angiotensin converting enzyme inhibition (ACEI) upon the progression from MA to overt diabetic nephropathy (DN). The aim of the present study was to evaluate the use of ACEI in type 1 diabetic patients with MA in an out-patient clinic, and the impact of ACEI on the progression of MA and the development of DN. **Materials and Methods:** During the first week of January 1995 we consecutively identified 217 type 1 diabetic patients with persistent MA (UAE between 30-300 mg/24 hours). All patients were followed up till 31. December 1998 or until death (n=8) or emigration (n=17). According to the development in MA before the 1. January 1995, the patients were divided into progressors (n=171, UAE≥100 mg/24 hours (n=154) or progression of UAE≥7 %/years (n=17)) or non-progressors (n=46, UAE < 100 mg/24 hours and progression of UAE < 7 %/years). According to new international guidelines all patients at high risk for the development of DN (progressors) were recommended treatment with ACEI. **Results:** Patients were followed up for a mean of 3.8 (range: 1-4) years. During follow up 75 % (128/171) of progressors and 37 % (17/46) of non-progressors were treated with ACEI ($p < 0.0001$). Mean rate of progression in UAE was -7 (95% CI: -13 to 0) %/yr in progressors and -13 (95% CI: -24 to -2) %/yr in non-progressors (p=0.4 between groups). DN developed in 19 % (32/171, ACEI treatment at diagnosis of DN, n=23) of the progressors, while none of the patients in the non-progressors developed DN. In 1998 46 % (78/171) of the so called progressors and 61 % (28/46) of non-progressors have regressed to normoalbuminuria ($p < 0.08$). **Conclusions:** Implementation of international guidelines concerning ACEI of microalbuminuria in diabetes reduced progression to diabetic nephropathy and enhanced regression to normoalbuminuria. This renoprotective effect was obtained despite only 75 % of at risk patients received ACEI.

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RENOPROTECTIVE EFFECTS OF ANGIOTENSIN II RECEPTOR BLOCKADE IN TYPE 1 DIABETIC PATIENTS WITH DIABETIC NEPHROPATHY.

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Aim: To compare the renal and haemodynamic effects of specific intervention in the renin-angiotensin system by blockade of the angiotensin II subtype 1-receptor (losartan) or by inhibition of the angiotensin I converting enzyme (ACE) (enalapril). **Material and Methods:** We performed a randomised, double-blind, cross-over trial in 16 type 1 diabetic patients (10 men), consisting of 5 periods each lasting 2 months. Patients received losartan 50 mg, losartan 100 mg, enalapril 10 mg, enalapril 20 mg and placebo in random order. At the end of each period albuminuria (ELISA), 24 hours blood pressure (TM2420 A&D) and glomerular filtration rate (GFR) (^{51}Cr-EDTA) were determined. **Results**: Placebo values of albuminuria (geometric mean (95% CI)), mean arterial blood pressure (MABP) (mean ± SEM) and GFR (mean ± SEM) were 1156 mg/d (643-2080), 104 ± 2 mm Hg and 90 ± 6 ml/min/1,73 m^2, respectively. Both doses of losartan and enalapril reduced albuminuria ($p<0.01$) and MABP ($p<0.02$), while GFR remained stable. Albuminuria was reduced by 33 % (95% CI (12-51)) on losartan 50 mg, 44 % (26-57) on losartan 100 mg, 45 % (23-61) on enalapril 10 mg and 59% (39-72) on enalapril 20 mg and MABP decreased 9 ± 2 (mean ± SEM), 8 ± 2, 6 ± 3 and 11 ± 3 mm Hg, respectively. No significant differences were found between the effects of losartan 100 mg and enalapril 20 mg. HbA1C and sodium intake remained unchanged, while a significant rise in serum potassium occurred during ACE inhibition. **Conclusion:** The angiotensin II subtype 1-receptor antagonist losartan reduces albuminuria and MABP similarly to the effect of ACE inhibition. These results indicate that the reduction in albuminuria and blood pressure during ACE inhibition are primarily caused by interference in the renin-angiotensin system. Our study suggest that losartan represents a valuable new drug in treatment of hypertension in type 1 diabetic patients with diabetic nephropathy.

OP 5
Retinopathy

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INCIDENCE OF DIABETES-RELATED BLINDNESS IN GERMANY 1990-1997

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Aims: A reduction of diabetes-related blindness by at least one third within five years was declared a primary objective in 1989. We collected data about incidence rates of blindness in a large district of Southern Germany (population: approximately 5 million) over 8 years to ascertain a potential change of incidence rates. **Methods:** We obtained complete lists of newly registered blindness allowance recipients (between 1990 and 1997) as well as age- and sex-specific population data of Württemberg-Hohenzollern, Germany. From these data we estimated age- and sex-specific and standardized incidence rates of blindness in the entire and the diabetic population (standard: estimated diabetic population 1989 in Württemberg-Hohenzollern). To test for time trend, we fitted a Poisson regression model stratified by diabetes using time difference from 1990 (years), age (categorized into 8 classes) and sex as independent variables. **Results:** There were 5 561 newly registered blindness allowance recipients (1990-1997). 3 733 (67%) were female, 1 545 (28%) had diabetes. Mean age was 72 years ± 21 (SD). Standardized results in the diabetic population (incidence rates per 100000 person-years; 95%-confidence-interval): 1990: 78(67;89); 1991: 83(72;95); 1992: 74(64;85); 1993: 71(61;81); 1994: 65(56;75); 1995: 78(67;88); 1996: 74(64;84); 1997: 65(55;74). The Poisson model estimated a 2.2% (95%CI:0.03;4.3%) decrease of incident blindness in the diabetic population per year (assuming linearity) that was marginally significant (p=0.047). No change could be observed in the total as well as in the non-diabetic population. **Conclusions:** Despite a marginally significant decrease in the Poisson model, no clear and substantial change of incidence rates could be observed over the past 8 years. To date, the objective of the St. Vincent Declaration has not been achieved. Specific interventions are needed to achieve a substantial reduction of diabetes-related blindness.

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CUMULATIVE GLYCEMIC EXPOSURE AND PROLIFERATIVE DIABETIC RETINOPATHY IN TYPE 1 DIABETES MELLITUS

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We have proposed that cumulative glycemic exposure (degree*duration) better distinguishes complication status in Type 1 diabetes than degree alone. **Aim**: To further analyze this issue the A1*months (A1M) methodology was applied to the public Diabetes Control and Complications Trial dataset. **Materials and Methods**: None of the Type 1 diabetic subjects (n = 1441) had proliferative retinopathy (PR) at baseline. They were followed for 5.7±4.2 years. A1M was calculated as the sum of (the months since diagnosis to the midpoint between eligibility and first trial HbA1c measurement multiplied by the HbA1c units above normal at eligibility) plus (# of months between the midpoints of the eligibility and first trial visit and the first and second trial visits multiplied by the HbA1c units above normal for the first trial visit) etc. up to the end of the study for non-cases, or up to the visit at which PR was diagnosed. **Results**: Cases had significantly higher duration (15.2±3.1 vs. 11.2±4.8 yr., P<0.0001), higher HbA1c at eligibility (10.2±1.7 vs. 9.0±1.6%, P<0.0001) and during the whole study (9.5±1.4 vs. 8.4±1.3%, P < 0.0001). The A1M value was also significantly higher among cases (687±272 vs. 329±217, P < 0.0001). The table shows the A1M and HbA1c values by duration for cases at onset of PR:

	<12.5 yr.	12.5-15 yr.	15-17.5 yr.	>17.5 yr.	P for linearity
A1M	613	600	746	769	NS
HbA1c	10.2	9.4	9.5	9.0	<0.05

A1M appeared more stabile across duration ranges among than the average HbA1c values, which showed a progressive decline. Using ROC curve analysis A1M had a significantly higher area under the curve than mean HbA1c (0.86 vs. 0.73, P<0.0001). **Conclusion**: A1M better identifies cases of proliferative retinopathy than mean HbA1c value.

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VITREOUS AND SERUM LEVELS OF ANGIOSTATIN IN PATIENTS WITH PROLIFERATIVE DIABETIC RETINOPATHY: ASSOCIATION WITH PREVIOUS RETINAL PHOTOCOAGULATION

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Retinal angiogenesis is a major characteristic of proliferative diabetic retinopathy (PDR) and is associated with increased levels of angiogenic growth factors such as VEGF and IGF-1. Scatter laser photocoagulation often prevents further retinal neovascularization and is associated with a reduction in the incidence of severe visual loss. However, it´s molecular mechanism is still unknown. Recently angiostatin, a fragment encompassing the kringle region of plasminogen, has been identified as a potent inhibitor of neovascularization and therefore is a potential mediator of the positive effects of retinal photocoagultion. **Aims:** This study investigates angiostatin production in proliferative diabetic retinopathy and the possible association with previous scatter laser photocoagulation. **Methods:** Serum and vitreous levels of angiostatin were determined in 18 control patients without retinal neovascularization, in 26 patients with PDR after scatter laser photocoagulation and in 6 patients with PDR without previous retinal photocoagultion. Vitreous was obtained on occasion of pars plana vitrectomy. Angiostatin was detected by western blotting using different specific antibodies. **Results**: Angiostatin could be detected in vitreous of 25 (of 32) patients with PDR, but only in 2 (of 18) control patients ($p < 0.0001$). 24 (of 26) patients with previous scatter photocoagulation had detectable angiostatin levels in vitreous compared to 3 (of 24) patients without previous photocoagulation ($p < 0.0001$). 1 (of 6) patient with PDR but without retinal photocoagulation had detectable angiostatin levels in vitreous compared to 24 (of 26) patients with PDR and previous photocoagulation ($p < 0.0001$). Only 2 patients had detectable angiostatin levels in serum. **Conclusions:** This study demonstrates a highly significant association between production of the angiogenesis inhibitor angiostatin in human vitreous and previous retinal scatter photocoagulation. Therefore angiostatin is a possible mediator of the positive effects of retinal photocoagulation. Regulation seems to occur locally, since angiostatin was not detected in relevant amounts in human serum.

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HEPATOCYTE GROWTH FACTOR IN VITREOUS OF PATIENTS WITH PROLIFERATIVE DIABETIC RETINOPATHY.

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Growth factors play an important role in neovascularization associated to proliferative diabetic retinopathy (PDR). Hepatocyte Growth Factor (HGF) is a powerful inducer of angiogenesis that stimulates proliferation and migration of endothelial cells. **Aim**: To determine whether HGF is elevated in the vitreous fluid of patients with proliferative diabetic retinopathy (PDR).**Materials and methods**: Serum and vitreous fluid samples were obtained at the time of vitreoretinal surgery from 17 patients with PDR, and 10 control nondiabetic patients with non proliferative ocular disease, matched by serum levels of HGF. Concentrations of HGF were determined by enzyme-linked immunoabsorbent assay (ELISA). Statistical analysis: the Mann-Whitney U test and Spearman´s rank correlation test. **Results**: Intravitreous concentrations of HGF (median and range) were significantly elevated in diabetic patients with PDR compared with nondiabetic patients [17,04 ng/ml (9,98-80) *vs.* 5,87 ng/ml (2,57-14,20); p<0,001]. Intravitreous concentrations of HGF were significantly higher than serum concentrations in diabetic patients [17,04 ng/ml (9,98-80) *vs.* 0,67 ng/ml (0,26-2,72);p<0,001] and also in the control group [5,87 ng/ml (2,57-14,20) *vs.* 0,71 ng/ml (0,49-0,96);p<0,001]. No correlation was found between serum and vitreous levels of HGF in both groups. **Conclusions**: Intravitreous concentrations of HGF are significantly higher in PDR. These results suggest that intraocular synthesis of HGF is involved in the neovascularization of PDR.

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ACCUMULATION OF 4-HYDROXYALKENALS IS AN EARLY MARKER OF OXIDATIVE STRESS IN THE DIABETIC RETINA

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Aims. Although oxidative stress is implicated in diabetes-induced pericyte loss and protein kinase C - mediated cascade leading to upregulation of VEGF and increased vascular permeability, the attempts to identify markers of retinal oxidative injury, especially in short-term diabetes, resulted in contradictory findings. This study was designed 1) to evaluate lipid peroxidation in early diabetic retina by the new method with N-methyl-2-phenylindol which, in contrast to TBARS, is specific for free malondialdehyde (MDA) and 4-hydroxyalkenals (4-HA); 2) to identify impaired anti-oxidative defense mechanisms; and 3) to assess if enhanced retinal oxidative stress in diabetes can be prevented by DL-α-lipoic acid (LA), the potent antioxidant penetrating through the blood-retinal barrier. Materials and methods: The groups included control (C) and STZ-diabetic (D) rats treated with or without LA (100 mg/kg/day i.p. for 6 wks). Total MDA plus 4-HA and MDA were measured after differential extraction. GSH was assayed with O-phthaldialdehyde, GSSG with GSSG reductase. Results: Total MDA plus 4-HA levels were increased in D (3.5± 0.96 vs 2.46±0.42 nmol/mg soluble prot in C, $p<0.05$), and this increase was prevented in D+ LA (2.23±1.11, $p<0.05$ vs.D). MDA and GSH levels were similar in C, D and D+LA (1.0±0.2, 1.2±0.2 and 1.1±0.2 nmol/mg soluble prot, and 18.7±3.4, 17.2± 3.5 and 18.5±3.7 nmol/mg total prot). Neither GSSG levels nor GSSG/GSH were different among the groups. Superoxide dismutase activity (SOD) was decreased 1.7-fold in D vs.C, and this reduction was prevented in D+LA. GSH peroxidase, GSSG reductase and GSH transferase activities were comparably decreased in D and D+LA vs. C. Catalase and cytoplasmic NADH oxidase activities were similar in three groups. Conclusions: Accumulation of α,β-unsaturated lipid aldehydes, 4-HA, but not MDA, is an early marker of oxidative stress in the diabetic retina. Increased lipid peroxidation occurs in early diabetes in the absence of GSH depletion, and is prevented by LA, potentially due to free radical scavenging and upregulation of SOD, without participation of the glutathione system.

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ANGIOTENSIN CONVERTING ENZYME INHIBITION AMELIORATES RETINAL OVEREXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND RETINAL HYPERPERMEABILITY

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Aims: Angiotensin converting enzyme (ACE) inhibition has been documented to have retino-protective actions in diabetic patients but the mechanism of this effect is unknown. Vascular endothelial growth factor (VEGF), is a permeability-inducing and endothelial cell mitogen which has been consistently implicated in the pathogenesis of both proliferative and nonproliferative diabetic retinopathy.

Materials and Methods: Thirty-six male Sprague Dawley rats were randomly assigned to receive streptozotocin (n = 24) or buffer (control, n = 12). STZ-diabetic rats were further randomized to receive the ACE inhibitor, perindopril (2 mg/l) in drinking water (n = 12). At 24 weeks, six rats in each of the 3 groups (control [C], diabetes [D], diabetes with perindopril [D+P]) underwent assessment of vascular permeability using a double isotope method. In the remaining 18 rats retinal VEGF mRNA was quantitated by *in situ* hybridization autoradiographic densitometry and grain counting within specific retinal layers.

Results: *In situ* hybridization autoradiographic densitometry revealed increased VEGF mRNA in the retinae of D compared with C (C: 166 ± 9 vs D: 252 ± 31 OD units, $p < 0.05$). This overexpression was reduced in D+P to levels similar to those of C (D+P: 101 ± 18 OD units, $p < 0.01$ D vs D+P). Similarly, by grain counting, increased VEGF mRNA was noted in the ganglion cell layer (GCL) and inner nuclear layer (INL) of D compared with C (GCL: 0.06 ± 0.01 vs 0.13 ± 0.02, C vs D, $p < 0.01$; INL: 0.03 ± 0.02 vs 0.12 ± 0.01, C vs D, $p < 0.01$). Perindopril treatment was accompanied by reduction in VEGF mRNA in both GCL and INL (GCL: 0.07 ± 0.02 and INL: 0.05 ± 0.01, $p < 0.01$ D vs D+P for both GCL and INL). Retinal permeability as indicated by the tissue:blood isotope ratio was increased in D (1.26 ± 0.03 vs 2.56 ± 0.32, C vs D, $p < 0.01$) and reduced in D+P (D+P: 1.47 ± 0.12, $p < 0.05$ vs D vs D+P).

Conclusions: Treatment of diabetic rats with ACE inhibition ameliorated diabetes-associated changes in both VEGF gene expression and vascular permeability.. These findings implicate the renin-angiotensin system in the VEGF overexpression which accompanies diabetic retinopathy and provide a potential mechanism for the beneficial effects of ACE inhibition in diabetic retinal disease.

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PROLIFERATIVE RETINOPATHY AND A NOVEL POLYMORPHISM OF THE GENE FOR THE ADVANCED GLYCATED RECEPTOR

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Aims: Formation of advanced glycation end products (AGEs) through covalent and irreversible non-enzymatic glycation of proteins or lipids are thought to play an important role in the pathogenesis of diabetic complications. AGE products are removed from the extracellular matrix through AGE receptors (RAGE). We have investigated RAGE as a candidate gene for proliferative retinopathy (PR).

Materials and methods: The regulatory region was sequenced and several novel polymorphisms identified including an imperfect trinucleotide repeat and 2 base pair insertion in the TATA box. A PCR-RFLP assay was developed for the latter using *Dra I* and agarose gel electrophoresis. The frequency of this polymorphism was compared in 119 type 2 diabetic South Indian patients with PR and 57 type 2 diabetic patients without diabetic retinopathy but at least 15 years disease duration (LTD).

Results: The RAGE genotype frequencies were significantly different between the groups (p=0.004: PR 20%DD, 80%Dd, 0%dd; LTD; 40%DD, 60%Dd, 0%dd). In order to replicate these findings we studied 61 families from the same ethnic group consisting of a proband with PR and at least 2 siblings; a sibling association test confirmed the case control study (p=0.005).

Conclusion: These studies suggest that polymorphism in the RAGE regulatory region may play an important role in the susceptibility to proliferative retinopathy.

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AMELIORATION OF RETINAL HEMODYNAMIC DYSFUNCTION USING A PROTEIN KINASE C β INHIBITOR IN PATIENTS WITH DIABETES.

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Aims: Abnormal retinal hemodynamics in preclinical models of diabetes have been attributed to the activation of protein kinase C β (PKC-β). To assess whether PKC-β activation occurred and affected retinal hemodynamics in humans, effects of LY333531, a PKC-β inhibitor, on retinal hemodynamics in patients with diabetes was assessed.

Materials and Methods: 29 patients with type 1 or 2 diabetes of less than 10 years duration and no or minimal retinopathy were entered into a 28 day double-masked, randomized, placebo-controlled, dose-ranging study. Patients received either placebo, or oral LY333531 doses of 8 mg twice a day, 16 mg once a day or 16 mg twice a day. Retinal blood flow (RBF) and mean retinal circulation time (MCT) were assessed by a fluorescein dye dilution technique at the beginning and end of the 28-day dosing period. Blood samples were collected for analysis of LY333531 and 338522, an equally potent and selective metabolite of LY333531, after the first and last doses.

Results: Increases in MCT and decreases in RBF are observed in patients with diabetes of less than 10 years duration and no or minimal retinopathy. Using a linear trend analysis across daily LY333531 doses, a statistically significant decrease was observed for MCT in patients receiving LY333531 (p=0.03). A trend toward a statistically significant increase in RBF was observed when RBF was correlated to LY333531 dose (p =.13). Similar relationships existed when RBF and MCT were correlated to the combined area under the curve (AUC) values of LY333531 and 338522, representing an index of exposure. Correlations with the rise in AUC values showed statistically significant linear relationships with a decrease in MCT (p=0.04) and an increase in RBF (p=0.004).

Conclusions: Oral administration of LY333531, an inhibitor of PKC β, ameliorates the abnormal retinal hemodynamics observed in patients with diabetes of less than 10 years in duration and having no or minimal retinopathy.

OP 6

Mechanisms of β-Cell Destruction and Regeneration

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INTERFERON-γ INDUCES INTERLEUKIN-1 CONVERTING ENZYME TRANSCRIPTION IN HUMAN ISLETS INDEPENDENTLY OF NITRIC OXIDE FORMATION.

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IL-1ß-induced nitric oxide (NO) production is crucial for rat β-cell destruction. Neither IL-1ß nor IFNγ alone induce NO production in human islets, and combinations of these cytokines lead to human β-cell apoptosis independently of NO. We recently analyzed transcription in human islets of the cysteine protease IL-1 converting enzyme (ICE), a caspase of key relevance in the apoptotic pathway. ICE expression was barely detectable in control and IL-1β -exposed islets, but there was a significant expression following exposure to TNFα+IFNγ and to IL-1ß+TNFα+IFNγ, however, at a lower level. Aim of the present study was to determine the effects of IFNγ or IL-1ß alone on ICE expression, and whether NO production is involved in this effect of cytokines. Material and Methods: Isolated human islets (from 3 different donors) were cultured for 6 or 24 h with IFNγ and IL1β separately or combined in the absence or presence of the iNOS inhibitor L-NMMA. RNA was isolated and multiplex RT-PCR analyses were performed. Results: ICE mRNA expression is expressed as percent of cyclophilin, used as internal standard. Results are mean +/- SEM.

	Control	IL-1β	IFNγ	IFNγ +IL-1β	IFNγ+IL-1β+LNMMA
6 h:	2.9 ± 1.1	1.6 ± 0.9	10.9 ± 3.2	5.9± 3.1	9.4± 2.2
24 h:	1.3 ± 0.6	1.3 ± 0.9	13.8 ± 2.7	7.3 ± 2.8	11.6±3.8

Conclusions: IFNγ alone, but not IL-1ß, induced ICE expression in human pancreatic islets independent of NO production. This induction was already present after 6 h ($p < 0.03$, t-test) and was maintained for 24h ($p < 0.02$). IL-1ß tended to inhibit IFNγ–induced ICE expression (6 h: $p = 0.045$ and 24 h: $p = 0.064$), an effect prevented by iNOS inhibition. It is thus conceivable that ICE expression is critically involved in cytokine-induced NO-independent human islet apoptosis.

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REDUCED CYTOKINE-MEDIATED CYTOTOXICITY IN BIOENGINEERED INSULIN-PRODUCING RINm5F CELLS THROUGH OVEREXPRESSION OF ANTIOXIDANT ENZYMES

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Aims: NO and reactive oxygen species (ROS) are crucial elements in cytokine-mediated ß-cell destruction. It was the aim of this study to characterise the cyto-protective effect of antioxidative enzyme overexpression against cytokine mediated cell toxicity. **Methods:** Catalase (CAT), glutathione peroxidase (Gpx) and Cu/Zn superoxide dismutase (SOD) were stably overexpressed in RINm5F cells. Cells were incubated with IL-1ß alone or with a combination of IL-1ß, TNF-α and IFN-γ for up to three days. Remaining viability was determined by the MTT-assay, cell proliferation rate by a BrdU-ELISA, the number of dead cells by counting and oxidative damage of cellular proteins was detected by a DNP-specific Western-blot. The cytokine-induced NO-production by iNOS was measured by the Griess-assay. **Results:** After a 72 h exposure of the cells to IL-1ß alone or a combination of IL-1ß, TNF-α and IFN-γ we observed a time- and concentration-dependent decrease of viability. While IL-1ß alone resulted only in a moderate reduction of viability in the range of 25 %, the cytokine mix revealed a drastic loss of viability of more than 60 %. Overexpressing cells were significantly protected against toxicity of the cytokine mix (remaining viability in % compared with untreated cells: Controls: 33±7, CAT: 61±4, Gpx: 71±6, and SOD: 60±4) but not against IL-1ß alone. The reduction of cytokine-mediated toxicity could also be confirmed by an increased proliferation rate and by a dramatic decrease of cell death. Overexpression of cytoprotective enzymes did not prevent the activation of iNOS after cytokine exposure. In contrast RINm5F cells overexpressing cytoprotective enzymes showed a significant lower level of ROS-damaged protein residues. **Conclusion:** The overexpression of cytoprotective enzymes protected insulin-producing cells against cytokine-mediated cytotoxicity, especially against ROS-mediated protein damage. Increasing the antioxidative defense status of insulin-producing cells with molecular biology techniques may pave the way to gene therapy strategies for prevention of ß-cell destruction during initial autoimmune attack in IDDM and after transplantation.

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Islet cell apoptosis and the development of overt IDDM in the spontaneously diabetic BB/S rat.

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The mechanisms underlying the progressive destruction of pancreatic B-cells leading to the development of IDDM remain unclear. The aim of this study was to investigate the mechanism of B-cell death in the spontaneously diabetic BB/S rat during the prediabetic period prior to onset of overt disease. Serial pancreatic biopsies were taken from cohorts of individual diabetes-prone (DP) and diabetes-resistant (DR) BB/S rats before (40 days), during (70 – 80 days) and after (112 days) development of diabetes. Paraffin sections of biopsy samples were screened for apoptosis (TUNEL) and Fas and Fas-L staining by light and confocal microscopy. No apoptotic cells were observed in the pancreas of any DR animals or DP rats aged 40 or 112 days. However, 4/7 DP rats biopsied at onset of IDDM showed intra-islet apoptosis with a further 2 animals showing apoptotic cells in non-islet tissue. Overall analysis revealed that >40% of detected apoptotic cells were located within islets and the apoptotic index (% of total islet cell number) for islet cells in the diabetic animals was approximately 3.5% (118/3595). Islet specific Fas staining was observed in several DP animals at 112 days but this was not associated with apoptosis. Fas-L staining was detected in endocrine and exocrine tissue in biopsies from DP rats both in the presence and absence of apoptosis. Thus these findings demonstrate a significant association between islet cell apoptsis and the development of overt IDDM in the BB/S rat. Furthermore, Fas expression does not appear to be involved in apoptotic B-cell death but a role for Fas-L cannot be excluded.

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NF-κB IS REQUIRED FOR CYTOKINE-INDUCED MANGANESE SUPEROXIDE DISMUTASE EXPRESSION IN INSULIN-PRODUCING CELLS

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Aims: Reactive oxygen species play an important role in the cytotoxic effect of inflammatory cytokines on pancreatic ß-cells in type I diabetes mellitus. The antioxidant enzyme manganese superoxide dismutase (MnSOD) is part of the cellular defenses against these deleterious radicals. MnSOD gene expression is induced by cytokines in insulin-producing cells but the transcriptional regulation of MnSOD expression in these cells is not well understood. In this study, we investigated the transcriptional regulation by cytokines of the rat MnSOD gene in an insulinoma cell line, RINm5F, and in FACS-purified rat beta-cells. **Materials and Methods:** We performed transient transfections with luciferase-reporter constructs containing the promoter region up to 2.5 kb and a 0.3-kb intron 2 fragment of the rat MnSOD gene, site-directed mutagenesis and band-shift assays following cell exposure to control condition, interkeukin-1ß (IL-1ß, 30 U/ml) and/ or interferon-γ (IFN-γ, 1000 U/ml) for 30 min to 16 h. **Results:** We identified two IL-1ß-responsive elements conferring each an additive three-fold IL-1ß-induced transcriptional activity ($p<0.001$ vs control, $n=3$ to 5) in both RINm5F cells and purified beta-cells. The first is located in the promoter region while the second is located in the second intron of the MnSOD gene. Interestingly, the intronic element is required for a two-fold IFN-γ-induced potentiation ($p<0.001$ vs IL-1ß, $n=5$) in RINm5F cells. Site-directed mutagenesis and band-shift assays showed that a NF-κB binding site in each region is necessary, but not sufficient, for transcriptional induction by IL-1ß. **Conclusions:** Our results suggest that NF-κB is required for cytokine-induced expression of MnSOD in rat insulin-producing cells and may cooperate with C/EBP factors binding in the promoter region and with factors binding to a C/EBP motif and to an Ets motif in the intronic region.

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IκB-α PHOSPHORYLATION INHIBITION AND IL-1ß INDUCED SUPPRESSION OF ISLETS

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Aims: The aim of this study was to examine if a novel inihibitor of TNF-α induced IκB-α phosphorylation, BAY 11-7082, could inhibit IL-1ß induced suppression of rat islet function. The transcription factor NF-κB is believed to be involved in IL-1 induced suppression of rat islet function. NF-κB is bound to its inhibitor, IκB, in the cytosol. Activation of NF-κB involves phosphorylation of IκB, followed by ubiquitination and degradation by the proteasome. This activates NF-κB, resulting in translocation to the nucleus, binding to specific DNA sequences followed by gene transcription. **Materials and Methods:** Rat islets were cultured in medium RPMI 1640 + 10% fetal calf serum and exposed for 48 h to 25 U/ml IL-1ß with the inhibitor (0.4, 4 and 10 μM) added 60 min before IL-1ß. **Results:** Pretreatment with BAY 11-7082 alone (0.4, 4 and 10 μM) had no suppressive effect on islet glucose oxidation (pmol/90 min x 10 islets; 220±24, 232±42 and 221±84). IL-1ß treated islets still showed a decrease in glucose oxidation (125±31, 91±33 and 107±32) when pretreated with the inhibitor (0.4, 4 and 10 μM). The inhibitor alone did not have any significant effects on insulin release (ng/h x 10 islets), neither at low (1.7 mM) or high (16.7 mM) glucose concentrations. IL-1ß induced suppression of glucose stimulated insulin release as compared to control (7±1.5 vs 24±5, $p<0.05$ vs control). This was partly counteracted by 10 μM BAY 11-7082 (19±3.3, $p<0.05$ vs IL-1ß treated islets). Lower concentration of the inhibitor was not effective. **Conclusions:** We conclude that our data points to the importance of IκB phosphorylation in IL-1ß induced suppression of islet insulin release.

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IDENTIFICATION OF A NOVEL CYTOKINE-INDUCED GENE IN ß-CELLS BY DIFFERENTIAL DISPLAY OF mRNA

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Aims: The cytokine IL-1ß is a potential mediator of ß-cell dysfunction and damage in type 1 diabetes mellitus. To clarify the molecular effects of this cytokine on ß-cells, we are presently searching for novel IL-1ß-induced genes. **Materials and Methods:** FACS-purified rat pancreatic ß-cells were exposed to control condition (no cytokines) or to IL-1ß (30 U/ml) for 6 or 24 h. Gene expression was compared by differential display of mRNA with RT-PCR (DDRT-PCR) and differentially expressed cDNAs were identified by sequencing. **Results:** Among the cytokine-induced genes, we identified a gene encoding for the rat serine protease inhibitor-3 (SPI-3). RT-PCR analysis confirmed that IL-1ß increases SPI-3 mRNA expression in rat ß-cells. IL-1ß induced a 5-fold ($P<0.05$ vs control) increase in SPI-3 mRNA content already after 2 h, with maximal expression at 6 h (12-fold; $P<0.05$) and decline after 24h. Similar observations were made in mouse pancreatic islets and in insulin-producing RINm5F cells. The stimulatory effects of IL-1ß on SPI-3 mRNA expression were decreased by actinomycin D (90% inhibition; $P<0.01$), by cycloheximide (70% inhibition; $P<0.01$) and by the NF-kB inhibitor PDTC (50% inhibition; $P<0.01$). A blocker of inducible nitric oxide synthase activity did not prevent IL-1ß-induced SPI-3 expression. **Conclusions:** SPI-3 is induced by IL-1ß in pancreatic ß-cells. This protein may be part of the "repair/defence" proteins triggered by ß-cells following immune-mediated damage. IL-1ß-induced SPI-3 mRNA expression in ß-cells depends on gene transcription, protein synthesis and activation of the transcription factor NF-kB, but not on NO production.

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SUSCEPTIBILITY AND DAMAGE OF FETAL RAT B CELL IN VITRO ARE INCREASED BY LOW PROTEIN DIET IN EARLY LIFE: PREVENTION BY TAURINE

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Background: We have previously shown that low protein diet (LP) in early life increased apoptosis in fetal and neonatal pancreatic ß cells *in vivo* and also decreased taurine concentrations in the fetal plasma. **Aim:** The principle objective of this study was, first, to verify the effect of LP diet during gestation on the sensitivity of fetal ß cells towards nitric oxide (NO) and secondly, to assess the protective effect of taurine, as an antioxidant in these conditions. **Methods:** Neoformed islets from fetuses of dams fed either LP (8%) or Control (20%) diet were incubated with taurine (0.3 or 3 mM) for 42 hours and then exposed to sodium nitropruside (SNP), a NO donor (at 10 and 100 μM) for the last 18 hours. BODIPY-FL dUTP, a specific probe of apoptosis, was used to detected apoptotic cell death using TUNEL method. In another series of experiments, the global cell death was measured by using ethidium bromide. In both experiments, confocal microscopy was used for the quantification of cell death. **Results:** Without any treatment, the number of ß cells positive for apoptosis were significantly higher in the LP islets (1.12 ± 0.33 %) than the C islets (0.3 ± 0.54 %, $P<0.001$). Addition of SNP at 100 μM showed an augmentation of the apoptotic rate in both groups but 12.14 ± 1.87 % of ß cells were apoptotic in C as compared to 19.15 ± 1.63 % in LP islets ($P< 0.001$). Beta cells of LP group are then more sensitive to NO than C group. The pre-treatment of islet cells with 3 mM taurine induced a significant diminution of apoptotic rate to 5.83 ± 1.29 % in the LP islets and to 2.18 ± 3.39 % in the C islets ($P<0.001$). Similar results were obtained when the global cell death was measured. **Conclusion:** Our results show that the LP diet in early life augments the susceptibility of fetal ß cells to NO. Moreover, taurine prevents this damage and could be envisaged in the prevention of type 1 diabetes. Its lower level in LP fetal plasma could contribute to the increased vulnerability of the LP fetal ß cell.

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IN UTERO MALNUTRITION IMPAIRS NEOGENESIS AND INCREASES APOPTOSIS IN THE BETA CELLS.

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Aims: Maternal food restriction during late pregnancy in rats decreases ß-cell mass in the offspring at birth. Prolonged maternal undernutrition until day 21 leads to 70% reduction in ß-cell mass (R group), although ß-cell proliferation remains normal. The aim of the present study is to further investigate the mechanisms controlling the expansion of ß-cell mass during postnatal malnutrition from birth to weaning. **Materials and methods:** Apoptosis was measured on pancreatic sections by the TUNEL method. Beta-cell regeneration was studied after streptozotocin treatment at birth (n0-STZ), a model involving increased neogenesis as well as proliferation of the remaining ß cells. Beta-cell mass and islet number quantified by morphometrical measurements on pancreatic sections in C-STZ and R-STZ rats, were compared to non-injected C and R rats, respectively. **Results:** In C animals we confirm in the ß cells a wave of apoptosis around day 14 postnatal. The number of apoptosing ß cells was significantly increased at all time points in R animals and peaked at day 12, in keeping with the stunting of ß-cell growth previously observed. STZ injection at birth induced hyperglycemia which normalized around day 7, both in T-STZ and R-STZ animals. In C-STZ rats, 80% of the ß cells were destroyed at day 4 and ß-cell mass regenerated to 50% that of non injected controls at day 7, with no further increase until day 21. The islet number/cm2 was entirely recovered from day 7, a reflect of increased neogenesis. However, in R-STZ animals, only 60% of the ß cells were destroyed at day 4 and ß-cell mass did not increase until day 21. Furthermore, the islet number/cm2 was half that of non injected animals throughout the study. **Conclusions:** *In utero* malnutrition impairs the capacity of postnatal ß-cell regeneration by neogenesis and increases apoptosis in the ß cells. These results provide an explanation for the impaired capacity of R animals to adapt ß-cell mass during ageing, which precipitates them in glucose intolerance.

OP 7

Experimental Aspects of Insulin Action

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PIVOTAL ROLE OF PPARγ IN ADIPOCYTE DIFFERENTIATION AND FAT ACCUMULATION: ANALYSYS OF PPARγ KNOCKOUT MICE

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Aims: We investigated the biological role of peroxisome proliferator-activated receptor γ (PPARγ) using PPARγ-deficient mice generated by gene targeting. **Methods and Results:** Heterozygous mutant mice (*PPARγ(+/-)*) were bred to generate homozygous mutant mice (*PPARγ(-/-)*). No *PPARγ(-/-)* were found in 65 live-born progeny of *PPARγ(+/-)* intercrosses, indicating that disruption of the PPARγ gene caused embryonic lethality. Viable *PPARγ(-/-)* were detected according to the Mendelian law until 10.5 d.p.c., but never detected later than 11.5d.p.c.. At 11.5d.p.c., however, we were not able to identify abnormalities by histological analysis of *PPARγ(-/-)* fetus. *PPARγ(+/-)* showed normal glucose tolerance and lipid metabolism under a normal diet. When wild-type (WT) and *PPARγ(+/-)* groups were fed with a high-carbohydrate diet, increases in body weight during 15 weeks were not distinguishable between the two groups. However, when they were fed with a high-fat diet, increase in body weight in WT was 22.5±1.4 g (n=13), whereas that in *PPARγ(+/-)* was 16.5±1.1 g (n=13) ($P<0.05$). Wet weight of white adipose tissues were 3.3±0.4g and 2.3±0.1g in WT and *PPARγ(+/-)*, respectively ($P<0.05$). To evaluate the role of PPARγ in adipocyte differentiation, we prepared primary embryonic fibroblast (EF) cells from *PPARγ(+/-)* intercrosses. Confluent EF cells were treated with the standard differentiation induction medium containing isobutylmethylxanthine, dexamethasone, insulin. The capacity of EF cells from *PPARγ(+/-)* to differentiate into adipocytes was lower than that from WT, and EF cells from *PPARγ(+/-)* were not able to differentiate into adipocytes. Lipid contents after 12days of differentiation were 85.6±6.1, 55.3±2.8, and 20.4±0.7 μg/mg of protein in EF cells from WT, *PPARγ(+/-)*, and *PPARγ(-/-)*, respectively (WT vs *PPARγ(+/-)*: $P<0.05$; *PPARγ(+/-)* vs *PPARγ(-/-)*: $P<0.01$). To evaluate the role of thiazolidindione in adopocyte differentiation, confluent EF cells were treated with the standard differentiation induction medium plus pioglitazone. While EF cells from *PPARγ(-/-)* failed to differentiate into adipocytes, the impaired capacity of EF cells from *PPARγ(+/-)* to differentiate into adipocytes was rescued by pioglitazone. **Conclusions:** This study provides the first direct evidence that PPARγ plays a pivotal role in fat accumulation and adipocyte differentiation.

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KRP-297 IMPROVES IMPAIRED FATTY ACID OXIDATION IN SKELETAL MUSCLES AND LIVERS OF ZUCKER DIABETIC FATTY RATS

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The α and γ isoforms of peroxisome proliferators-activated receptors (PPARs) are key regulators in lipid homeostasis. We recently reported that a novel thiazolidinedione (TZD), KRP-297 (K) was a ligand for PPARα and PPARγ, unlike classical TZDs such as troglitazone (T), were PPARγ-specific ligands. To understand the role of PPARα and PPARγ on lipid abnormalities in insulin resistance, K (10 mg/kg) and T (300 mg/kg) were administered orally to insulin-resistant Zucker diabetic fatty (ZDF) rats for 2 weeks, and CO_2 production from palmitic acid (PA, C16:0) in liver, soleus muscle, and epididymal adipose tissue were measured. **Results:** PA oxidation in soleus muscles was lower ($P<0.05$) in ZDF rats (3.97±0.45 nmol/g) than in lean (L) rats (9.12±1.46 nmol/g). Decrease in PA oxidation in the soleus muscles from ZDF rats was improved by K (7.56± 1.08 nmol/g, $P<0.05$) and T (7.20±1.04 nmol/g, $P<0.05$). Hepatic PA oxidation was also lower ($P<0.05$) in ZDF rats (84±13 pmol/DNA) than in L rats (190±19 pmol/DNA). The defect in PA oxidation in the livers of ZDF rats was improved ($P<0.05$) by K (159±25 pmol/DNA), unlike by T (98±0.6 pmol/DNA). The defect in PA oxidation in the livers from ZDF rats was not observed when the C8-fatty acid was used as a substrate, unlike the C16 and C18-fatty acids. There was no significant difference between ZDF and L rats in PA oxidation in adipose tissues, and K and T had no influence on PA oxidation in these tissues. **Conclusion:** K improved reduced fatty acid oxidation in the livers and skeletal muscles of ZDF rats, while T had an effect only in the skeletal muscles. These results suggest that agonisms of PPARα and PPARγ may play a role in the prevention of lipid accumulation causing insulin resistance (lipotoxicity) in the skeletal muscles and livers.

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EARLY GROWTH RETARDATION LEADS TO ADIPOCYTE INSULIN RESISTANCE.

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Many studies have revealed a relationship between early growth retardation and Type 2 diabetes. Aim: To investigate the effect of early growth retardation on adipocyte insulin action in late adult life. Methods: Rats were fed either a 20 % or an 8 % protein diet during pregancy and lactation. Offspring were weaned onto a 20 % protein diet and studied at 15 months of age. Results: Adipocytes from growth retarded offspring (LP) (of dams fed 8 % protein diet) had elevated basal glucose uptake compared to control offspring (C) (mothers fed 20 % protein diet) (104 + 5 compared to 79 ± 7 amol/min/cell, $p < 0.05$). Insulin stimulated glucose uptake into C adipocytes (121 ± 9 amol/min/cell) but had no effect on LP adipocytes (106 ± 7 amol/min/cell). Basal rates of lipolysis (5.85 ± 0.67 and 5.18 ± 0.80 nmol glycerol released/h/10^4 cells for C and LP respectively) were similar as were isoproterenol stimulated rates (9.10 ± 0.99 and 8.25 ± 0.73 nmol glycerol released/h/10^4 cells for C and LP respectively). Insulin inhibited this stimulated release in C adipocytes (4.93 ± 0.46 nmol glycerol released/h/10^4 cells) but had a reduced effect on LP adipocytes with rates above basal (7.23 ± 0.68 nmol glycerol released/h/10^4 cells, $p < 0.01$). These differences were not related to changes in insulin receptor expression but were linked to decreased insulin stimulated phosphotyrosine-associated phosphatidylinositol 3-kinase activity in LP adipocytes (4.5 ± 0.7 fold increase in LP versus 7.5 ± 0.6 fold increase in C, $p < 0.05$). Conclusions: Maternal protein restriction leads to adipocyte insulin resistance with a molecular basis downstream of the insulin receptor in the insulin signalling pathway.

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GLYCOGEN SYNTHASE AND PHOSPHORYLASE ACTIVITY IN SKELETAL MUSCLE AND LIVER OF HYPERINSULINAEMIC RATS.

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Aim: The time dependent regulation of muscle and liver glycogen synthesis by insulin was examined in conscious rats. **Materials and Methods**: A hyperinsulinaemic (~600 pmol/l) euglycaemic (5.5 mmol/l) clamp with 3-3H-glucose infusion. After 0, 0.5, 2, 4, 8 or 12 h of hyperinsulinaemia, rectus abdominus/psoas muscles and liver were freeze-clamped for in vitro assay of glycogen synthase (GS) and phosphorylase (GP) activity, glycogen and glucose-6-phosphate (G6P) concentrations and 3-3H-glucose incorporation in glycogen. All reported changes are $p<0.05$. **Results:** **Skeletal muscle:** within 0.5-4 h of hyperinsulinaemia, GS fractional activity (sensitivity to G6P) increased from 18±2 to 35±3%; thereafter desensitization occurred and after 12 h of hyperinsulinaemia GS activity decreased to baseline values (17±3%). GP activity (ratio between GPa and GPb) decreased from 64±5 to 32±4% within 2-4 h of hyperinsulinaemia and remained suppressed thereafter. Muscle glycogen synthesis correlated with in vitro GS activity but GP activity correlated inversely with glycogen synthesis only during the initial 4 h of hyperinsulinaemia. Thereafter muscle glycogen synthesis decreased to baseline values whereas GP activity remained suppressed. Intracellular G6P concentrations steadily increased from 0.32±0.07 to 0.79±0.11 μmol/g muscle during the 12 h of hyperinsulinaemia confirming the insensitivity of GS to G6P. **Liver:** within 0.5-2 h of hyperinsulinaemia, GS activity increased from 25±2 to 40±4% and GP activity decreased from 30±4 to 17±4% and remained constant for 12 h. Liver glycogen synthesis correlated with in vitro GS and correlated inversely with GP activity during the initial 8 h of hyperinsulinaemia. Thereafter (8 to 12 h) liver glycogen synthesis increased 3-fold (from 1.0±0.3 to 3.2±0.9 mg/kg.min), whereas in vitro GS and GP activity remained unchanged. Intracellular G6P concentrations increased from 0.17±0.02 to 0.36±0.03 μmol/g liver from 8 to 12 h of hyperinsulinaemia which may explain the increase in in vivo liver glycogen synthesis. **Conclusion:** Long-term hyperinsulinaemia induces desensitization of GS but not GP activity in skeletal muscle; in liver, no desensitization of GS and GP activity occurs.

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INSULIN RESISTANCE AND THE MALONYL-CoA FUEL SENSING MECHANISM IN THE hHTg RAT

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Aims: It has been proposed that an increase in the cytosolic concentration of long chain fatty acyl coenzyme-A (LCFA CoA) in skeletal muscle plays a role in the pathogenesis of insulin resistance (IR). Recent studies suggest that high levels of circulating free fatty acids (FFA) and muscle triglycerides (Tg) and malonyl CoA may be important contributing factors. To test this hypothesis, the levels of these "metabolites" were measured in a non-obese rodent with IR, the hereditary hypertriglyceridemic (hHTg) rat. **Materials and methods:** Control (C) and hHTg rats were fed a basal chow diet (BD) or a diet containing 70 cal% fat (HF), which has previously been shown to produce IR. Insulin action was assessed during an euglycemic hyperinsulinemic clamp (6.4 mU/kg/min) plus ^{3}H-2-deoxyglucose tracer administration. **Results:** The levels of serum FFA (0.89±0.12 vs 0.47±0.08 mmol/l) and malonyl-CoA (4.8±0.8 vs 2.3±0.2 nmol/g), and Tg in muscle (3.5±0.3 vs 1.7±0.4 ±µmol/g) were significantly higher in the hHtg than in C rats. When fed the HF diet, the concentration of malonyl CoA (4.3±0.6), Tg (5.1±0.4) and FFA (1.1±0.1) all increased in controls. In contrast, no changes in the already high levels of these metabolites were observed in hHTg rats. Insulin resistance, as evidenced by glucose infusion rate (GIR) during the clamp, was present in the hHTg rats on the basal diet (C-BD: 28.4±5.8 vs hHTg-BD: 20.2 ±0.4 mg/kg/min; $p<0.001$), but was not diminished further by the HF diet (hHTg-HF: 18.6 ±0.6). In contrast, the HF diet decreased GIR by 50% in C (15.2 ±0.4). In keeping with these findings, the glucose metabolic index (Rg') in skeletal muscles (Q. femoris: C-BD: 25.6±1.5 vs C-HF: 12.3±1.1 mmol/100 g /min; $p<0.001$) was reduced by HF diet in C animals, but not in the hHTg rats (hHtg-BD: 15.7±1.2 vs hHTg-HF: 17.5± 2.0). **Conclusions:** The results show that increased concentrations of malonyl CoA and Tg in skeletal muscle are associated with insulin resistance with both the hHTg rat and control rats fed a HF diet. They also reveal that the same HF diet does not worsen IR or increase the already high levels of Tg or malonyl CoA in muscle of the hHTg rat. Whether these changes in muscle lipids are the cause or result of the insulin resistance remains to be determined.

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EFFECTS OF CHRONIC GLUCOSAMINE ON IN VIVO GLUCOSE METABOLISM.

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Aims: In vitro experiments suggest that increased glucose flux through the hexosamine pathway may be responsible for glucose toxicity. We and others recently demonstrated that an *acute* infusion of exogenous glucosamine determines insulin resistance in normal rats in vivo, possibly through the inhibition of glucose transport. Glucose toxicity, however, is a *chronic* phenomenon. Aim of this study was to evaluate the effect of a chronic glucosamine infusion in control rats. **Methods**: We studied four groups of animals: Diabetic (90 % pancreatectomy) rats (D, n=6), Control rats chronically (7 days) i.v. infused with glucosamine (5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; CG, n=6) through an overhead suspended swivel, Control rats chronically i.v. infused with saline (CS, n=5), Control rats *acutely* infused with glucosamine (5 $\mu \cdot mol \cdot kg^{-1} \cdot min^{-1}$; AG, n=5). Chronic i.v. glucosamine was substituted with saline 24h prior the study. All animals were fasted for 24h, and insulin-mediated glucose metabolism was evaluated with euglycemic hyperinsulinemic (20 $mU \cdot kg^{-1} \cdot min^{-1}$) clamps (+[3-^{3}H]-glucose). **Results**: Acute glucosamine reduced glucose uptake by ~25% (AG: 206±12 vs. CS: 271±16 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; $p<0.01$). Whole body glycolysis (calculated from the ^3H_2O rate of appearance) and muscle glycogen synthesis were similarly reduced, both contributing to the overall glucosamine-induced insulin resistance, as previously reported. Diabetic animals (made similarly euglycemic by the 24h fast) resulted markedly insulin resistant (D: 177±16 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; $p<0.001$) with a reduction in glycogen synthesis mostly contributing to the reduced glucose uptake. Finally, rats chronically infused with glucosamine resulted similar to the saline infused rats, both when comparing overall glucose uptake (CG: 255±18 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) or glycogen synthesis and glycolysis. **Conclusions**: It is therefore concluded that increased hexosamine metabolism in-vivo: 1) when acutely increased (during the clamp) reduces glycolysis and glycogen synthesis, with both pathways contributing to the decreased glucose uptake; 2) if the same increase of the hexosamine is prolonged for 7 days, and then discontinued for 24h, normal insulin sensitivity is restored. Since diabetic animals are still insulin-resistant in presence of (a shorter) euglycemia, 3) either a more (than 7 days) prolonged glucosamine infusion or a shorter (than 24h) interruption is needed to mimic glucose toxicity through a glucosamine infusion.

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GLUT-4 OVEREXPRESSION INCREASES GLUCOSE TRANSPORT IN L6 CELLS AND PRIMARY HUMAN MYOBLASTS FROM NIDDM PATIENTS

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A reduced glucose transport (GT) into skeletal muscle is the hallmark of insulin resistance in type II diabetes. Data from transgenic animals show that Glut-4 muscular overexpression corrects the diabetic phenotype. Aim of the study was the correction of GT in primary human myoblasts from type II diabetic patients by Glut-4 overexpression. To this end, we have generated a retroviral vector carrying the human Glut-4 cDNA (a gift of GI Bell) under the Moloney Murine Leukemia Virus LTR and the truncated, non-functional human nerve growth factor receptor (NGFr) for selection of transduced cells under the SV40 promoter. This construct was initially validated on L6 myoblasts, a rat skeletal muscle cell line. After transduction, these cells showed a 75% positivity for NGFr expression by FACS scan, and the expression of Glut-4 by immunofluorescence analysis. A significant increase in both basal and insulin-stimulated glucose transport was demonstrated after transduction: (pmol.min^{-1}.mg protein^{-1}) myoblasts: basal: from 184±66 to 417±84, stimulated: from 226±88 to 582±135; myotubes: basal: from 270±79 to 602±122, stimulated: from 414±111 to 1521±452). Primary human myoblasts from NIDDM patients, which retain a defect of glucose transport in vitro, are being isolated and expanded in vitro. These cells have been transduced with high efficiency (100%) after 3 infection cycles. Preliminary data on these cells are in agreement with data obtained in L6 cells, showing a 2 to 3-fold increase in specific glucose transport, both in the basal as well as in the insulin-stimulated condition. These data might constitute the basis for a gene therapy approach to NIDDM in humans.

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RAB4 AND RAB11 REPRESENT TWO POTENTIAL MEDIATORS OF GLUT4 DISTRIBUTION AND TRANSLOCATION IN THE HEART

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Aims: Earlier investigations from this laboratory demonstrated the presence of a 24 kDa GTP-binding protein in cardiac GLUT4-vesicles. Western blotting analysis indicated that Rab4 (24 kDa) is expressed in the heart but does not colocalize to GLUT4. The functional implications of Rab4 and p24 in GLUT4 translocation were investigated using an insulin-sensitive cardiac cell line (clone K6) stably overexpressing GLUT4. **Methods:** Cells were transiently transfected (efficiency 60-70%) with Rab4-cDNA or Rab3-cDNA and glucose transport and GLUT4 translocation were determined. **Results:** Rab4 but not Rab3 reduced basal glucose transport with an enhancement of insulin action by 30-40%. Cell surface biotinylation indicated that Rab4 overexpression resulted in a reduced GLUT4 abundance (about 40%) at the cell surface in basal cells. Insulin increased cell surface-GLUT4 by 100% compared to only 26% in control cells. In order to identify the p24 in the GLUT4 vesicles, a cholate extract from a crude membrane fraction of pig heart was subjected to a three-step FPLC purification protocol. Fractions were analysed for the presence of 24 kDa small GTPases, finally pooled and submitted to two-dimensional electrophoresis. Three spots of interest were analysed by mass spectrometry and identified as Rab11. Western blot analysis of microsomal membranes from pig and rat heart confirmed the expression of Rab11. Furthermore, GLUT4-vesicles isolated by immunoadsorption from cardiac microsomes were found to contain an appreciable amount of Rab11, which increased in response to insulin. **Conclusions:** Our data show that Rab4 is involved in directing GLUT4 to the insulin-sensitive intracellular compartment. Rab11 colocalizes to GLUT4 and may be involved in exocytosis and/or recycling of the transporter. We suggest that at least two Rab proteins with different intracellular locations could represent essential parts of the GLUT4-translocation machinery.

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OP 8

Recent Progress in Insulin Therapy

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EFFECTS OF THE RAPID-ACTING INSULIN ANALOGUE INSULIN ASPART ON POSTPRANDIAL GLYCAEMIC EXCURSIONS IN TYPE 2 DIABETES

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Aims: The aim of the present study was, in a double blind, double dummy crossover design to compare the postprandial serum glucose profile after Insulin Aspart (IAsp) 0.15 U/kg body weight given at time t=0 minutes, to the postprandial serum glucose profile after Actrapid 0.15 U/kg given at time t=0(Act_0) and t=-30 (Act_{30}) minutes in relation to a test meal in patients with insulin treated Type 2 diabetes. We also wanted to study individual responses in absorption of IAsp using a new and specific monoclonal assay. Material and methods: Twenty-five (14M/11F) Type 2 patients with a mean age of 59.7 (range 43-71) years, Body Mass Index (BMI) 28.3 (21.9-35.9) kg/m^2, HbA_{1c} 8.5 (6.8-10.0)%, glucagon stimulated C-peptide 0.6 (0.3-2.5) nmol/l and diabetes duration 12.5 (3.0-26.0) years participated. We determined the total excursion of serum glucose concentration (EXC_{glu}) 0-360 min calculated as the incremental area under the curve. We also measured maximal concentration C_{max}IAsp, time to maximal concentration t_{max}IAsp and $t_{1/2}$ IAsp with the specific assay. Results: Mean (SD) EXC_{glu} was significantly smaller for IAsp 899(± 609) mmol/l x min compared to Act_0 1101 (± 497) mmol/l x min ($p = 0.01$). No difference in EXC_{glu} could be demonstrated when comparing IAsp to Act_{30} 899 (± 609) mmol/l min vs 868 (± 374) mmol/l x min. Furthermore, maximum serum glucose concentration was significantly ($p<0.02$) lower for IAsp 10.8 ± 2.2 mmol/l compared to Act_0 12.0 ± 2.4 mmol/l. The median (range) C_{max}IAsp was 50.3 (11.0-164.8) mU/l, t_{max}IAsp 60.0(20-180) minutes and $t_{1/2}$ IAsp 76(40-194) minutes. Conclusion: Immediate pre-meal administration of IAsp in patients with Type 2 diabetes resulted in improved postprandial glucose control compared to Actrapid injected immediately before the meal, but similar control compared to Actrapid injected 30 minutes before the meal. The absorption of IAsp is variable and slower in Type 2 than previously reported in Type 1 diabetic patients.

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TEMPORAL EFFECT OF ACUTE ELEVATIONS IN FREE FATTY ACIDS ON ACTION OF FATTY ACID ACYLATED INSULIN, NN304.

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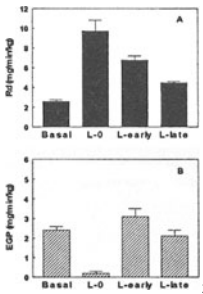
Binding of myristic acid acylated insulin, NN304, to fatty acid binding sites on albumin results in prolongation of NN304 action. Aims: This study examined the temporal effect of acute elevations in free fatty acids (NEFA) on NN304 action. A rise in plasma NEFA levels was initiated either with NN304 or after establishing NN304-stimulated glucose turnover. Methods: Three protocols were employed during euglycemic clamps in normal dogs (somatostatin; and 3.6pmol/min/kg i.v. NN304 infusion for 360 min): **L-0**(no Liposyn; n=13), **L-early**(-30 to 360 min Liposyn, 1.5 ml/min; n=7), **L-late**(180 to 360 min Liposyn; n=7). Results: Plasma steady state total NN304 (bound plus free) levels were 2517±108 and 2249±152pM for L-0 and L-late, and 1728±87pM for L-early ($p<0.01$) consistent with competitive NN304 displacement from albumin when NEFA and NN304 rise simultaneously. Basal NEFA (0.8±0.1mM) fell to 0.18±0.02 mM with L-0, and were raised to 2.9±0.2 and 2.6±0.1mM in L-early and L-late. NN304 alone (L-0) acted equally to stimulate glucose uptake (Rd; 3.4-fold increase, Fig.A) and suppress glucose production (EGP; 92% suppression, Fig.B). Elevating NEFA early (L-early) caused a 30% reduction in stimulation of Rd and total reversal of suppression of EGP ($P\leq0.05$vs.L-0). Initiating a late rise in NEFA (L-late) caused a greater reduction in Rd (54%;$P<0.01$), and similar reversal of suppression of EGP ($P>0.05$vs.L-early). Conclusions: 1) Like native insulin, NN304 acts indirectly to modify EGP via changes in lipolysis (NEFA); 2) The NEFA effect on Rd is less with an early vs. late NEFA rise due to NN304 displacement from albumin when NEFA and NN304 rise concurrently. During NN304 administration, the potent effect of NEFA to induce insulin resistance is influenced by the timing of elevation in plasma NEFA.



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CSII *VERSUS* MDI IN IDDM PATIENTS TREATED WITH INSULIN LISPRO: RESULTS OF A RANDOMISED, CROSS-OVER TRIAL

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Aims: To compare the efficacy of programmable external pump (CSII) with insulin lispro (LP) and multiple daily injections (MDI) with LP on diabetes control of type 1 diabetic patients previously on CSII with regular insulin (RI). ***Materials and methods:*** Forty-one, C-peptide negative, type 1 diabetic patients (sex: 21M/20F; age: 43 ±10yrs; diabetes duration: 20 ±11yrs; BMI: 24.0 ± 2.4kg/m^2; HbA1c: 8.39 ±.87%) participated in a randomised, cross-over trial. After a 6 weeks period of CSII with RI, they were randomly assigned either to CSII with LP or to MDI with LP(before meals) and NPH(morning and bedtime) for a 4 months period. Then, they were switched to the other treatment for another 4 months. The patients were advised to check capillary blood glucose 6 times a day. Instructions were given to investigators to rapidly optimise insulin regimens by introducing a third NPH injection at noon if necessary. At the end of each period, HbA1c was measured and pâtients' memory meters were down-loaded to collect the mean glycemia, the mean standard deviation of glycemia, an index of glycemic fluctuations, and the number of hypoglycemia during the previous 2 weeks. ***Results:*** Forty patients completed the study. One patient dropped out 3 weeks after randomisation during MDI period. Basal insulin regimen had to be optimised in 75% of the patients during the MDI period (mean n of NPH injections/d : 2.65). The table shows the metabolic results observed at the end of each sequence of treatment (analysis of variance for cross-over study). Results are expressed as mean±SD.

	CSII	MDI	p
HbA1c (%)	7.89 ±.77	8.24 ±.77	<0.001
mean Glycemia/14days (mg/100ml)	165 ±27	175 ±33	<0.05
mean SD glycemia/14days (mg/100ml)	73 ±15	82 ±18	<0.01
glycemia<60mg/100ml/14days (n)	3.9 ±4.2	4.3 ±3.9	NS
Mean insulin doses(IU/d)	38.5 ±9.8	47.3 ±14.9	<0.0001

Conclusions: This randomised study demonstrates the superiority of CSII over MDI with insulin Lispro in terms of glycemic control and stability in type 1 diabetic patients, with lower insulin daily doses.

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INSULIN ASPART IMPROVES LONG-TERM GLYCAEMIC CONTROL IN TYPE 1 DIABETES - A RANDOMIZED TRIAL VS. HUMAN INSULIN

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Aims: Insulin aspart is a rapid-acting insulin analogue to be administered immediately before meals, instead of 30 min before meals as is recommended for human insulin. The present study was performed to evaluate blood glucose control over 6 months with insulin aspart versus human insulin as meal-related insulin. **Materials and Methods:** People with Type 1 diabetes were randomized to 6 months treatment with insulin aspart (n=707) or human insulin (n=358) as meal-related insulin three times daily with once or twice daily basal NPH insulin. Insulin aspart was given immediately before meals, and human insulin 30 min before meals. Efficacy and safety were evaluated after 6 months treatment. **Results:** At 6 months insulin aspart was superior to human insulin with respect to glycaemic control. Baseline-adjusted HbA_{1c} was 0.12 (95%-CI 0.03 – 0.22) %Hb lower in the insulin aspart group than with human insulin ($p<0.02$). Prandial glucose increment over the three meals decreased in the insulin aspart group while remaining unchanged in the human insulin group, the difference at 6 months being 1.15 (0.87 - 1.43) mmol/l ($p<0.0001$). The dose of pre-prandial insulin aspart and human insulin remained unchanged over the treatment period, while the NPH insulin dose was 8 % higher at 6 months in the insulin aspart group than with human insulin. The risk of experiencing hypoglycaemia requiring third party intervention with insulin aspart relative to human insulin was 0.83 (0.59-1.18) (NS), while the risk of any symptomatic hypoglycaemia did not differ between groups. Major nocturnal hypoglycaemic events requiring parenteral treatment were fewer with insulin aspart (1.3 vs 3.4 % of patients) ($p<0.05$). Treatment satisfaction as assessed by the WHO Diabetes Treatment Satisfaction Questionnaire was significantly improved with insulin aspart with a difference of 2.3 (1.2 – 3.3) points ($p<0.0001$). **Conclusions:** Insulin aspart gave improved overall blood glucose control, fewer severe night-time hypoglycaemic events, and improved satisfaction when compared with human insulin.

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BASAL INSULIN GLARGINE (HOE 901) IN TYPE 2 DIABETES:
A 28-WEEK, NPH CONTROLLED TRIAL.
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Aims: Insulin glargine (21^{A}-Gly-30^{B}a-L-Arg-30^{B}b-L-Arg-human insulin) is a biosynthetic insulin analog with a prolonged action compared to NPH. This multicenter, randomized, parallel group study compared insulin glargine with NPH in subjects with type 2 DM treated with insulin therapy without oral antidiabetic agents. **Materials and Methods:** Subjects were treated with insulin glargine once daily (bedtime), NPH once daily (bedtime) or NPH twice daily (morning and bedtime) and were allowed to use preprandial regular insulin as part of the daily regimen. **Results:** A total of 518 subjects with a mean age 59.3 years, mean glycohemoglobin (GHb) 8.5%, and mean fasting plasma glucose (FPG) 10.9 mmol/L were treated at 59 centers for up to 28 weeks. Total daily insulin dose at baseline, mean ± SD, was 64 ± 32 IU. There was no change in the basal dose of insulin glargine from baseline to endpoint, (-1 ± 19 IU, NS), whereas the dose of NPH was increased (7 ± 19 IU, $p<0.01$). Regular insulin use increased in both the insulin glargine and NPH groups (10 ± 22 IU vs. 6 ± 16 IU, respectively, $p<0.01$). GHb was reduced ($p<0.01$) similarly from baseline in the insulin glargine and NPH treated groups (-0.41% vs. -0.59%, respectively, N.S.). Compared to the respective baselines, insulin glargine and NPH decreased FPG at endpoint ($p<0.01$) by –1.73 mmol/L vs. –1.08 mmol/L, respectively (NS). Weight gain at endpoint was greater in the NPH group (0.4 kg vs. 1.4 kg, $p<0.01$). The number of subjects experiencing symptomatic nocturnal hypoglycemia was less ($p<0.02$) with insulin glargine (31.3%) than NPH (40.2%). Fewer subjects experienced severe hypoglycemia (insulin glargine 0.4% vs. NPH 2.3%, $p=0.058$). The overall adverse event profiles of insulin glargine and NPH were similar, although a slightly higher incidence of mild injection site pain occurred with insulin glargine. **Conclusions:** Once daily basal insulin glargine (n=246) was as effective as once (n=48) or twice (n=207) daily NPH in improving glycemic control with less symptomatic nocturnal hypoglycemia.

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INSULIN REGIMENS AND METABOLIC CONTROL IN ADOLESCENTS WITH TYPE 1 DIABETES OVER 3 YEARS.
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Aims: From the total number of 2873 children and adolescents included in an international study in 1995, 892 adolescents were restudied in 1998 including clinical parameters and HbA_{1c} centrally measured. **Material and methods:** There were 441 boys and 451 girls, mean age 11.3 ± 2.2 years and mean diabetes duration 4.6 ± 3.0 years in 1995. Only children with a diabetes duration of one year or more (1995) were considered thus excluding most children in the remission phase. All were treated by the same departments during the study period. **Results:** Over the 3-years metabolic control as assessed by HbA_{1c} deteriorated from 8.7 ± 1.6% in 1995 to 8. 9 ± 1.6 in 1998 despite the increasing use of multiple injection regimens (three or more) from 42 to 70%. The study group was divided into three subgroups according to injection regimen in 1995 and in 1998. Group 1 (n=250) remained on twice-daily insulin; group 2 (n=366) remained on multiple injections (three or more) ; group 3 (n=256) shifted from twice-daily insulin to multiple injections from 1995 to 1998. During this period the mean HbA_{1c} level increased 0.3 % in group 1, 0.2 % in group 2 and 0.2 % in group 3. However the changes were lower than expected with regard to the known effects of age and duration. The increase in daily insulin dose between 1995-98 was significantly higher in group 3 (0.17 U/kg/24h compared to group 2 (0.09 U/kg/24h) . Body mass index (BMI) increased significantly in all subgroups during the study period. The BMI change was significantly higher for females in group 3 (3.6 kg/m^2) compared to females in group 2 (2.8 kg/m^2). Twice daily premixed insulin alone was used in 21.7% of the adolescents in 1995 and 13.5% in 1998. Those remaining on twice daily premixed insulin over the three year period showed similar HbA_{1c} values compared to those shifting to another regimen during the period (p=0.9). **Conclusion:** In this large international cohort of adolescents metabolic control is unsatisfactory irrespective of insulin injection regimen. Other factors must be important.

OP 9
Lipids

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Plasma lipids and hypoglycaemic therapies over 6 years in Type 2 diabetic patients in the UKPDS
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Aims : We have examined patients included in the UKPDS to determine whether intensive treatment with hypoglycaemic agents alters lipid profiles compared with a non-intensive policy. **Patients :** 2249 newly diagnosed White Caucasian Type 2 diabetic patients : 57% male; mean (SD) age 54 (8) years; BMI 27.9 (5.3) kg/m^2; systolic/diastolic BP 138/84 (19/10) mmHg; total cholesterol (***TC***) 5.5 (1.1) mmol/L; ***LDL*** cholesterol 3.6 (1.1) mmol/L; ***HDL*** cholesterol 1.07 (0.23) mmol/L; geometric mean (1SD interval) triglyceride (***TG***) 1.6 (1.0, 2.7) (mmol/L); median (IQR) fasting plasma glucose 8.3 (7.3, 10.0) mmol/L; HbA_{1c}7.0 (6.1, 8.1) %. **Therapies :** randomly allocated; conventional policy, primarily diet alone; intensive policy : chlorpropamide; glibenclamide; insulin or metformin (overweight patients). **Analysis** : intention to treat. **Results** : Over 1 year ***TC*** and ***TG*** increased with conventional policy while intensive policy prevented this increase. The effect was particularly apparent for chlorpropamide with ***TC*** (mean difference (95%CI)) -0.15 (-0.31, -0.01) mmol/L, and ***TG*** -0.22 (-0.41, -0.03) mmol/L, but was not as marked with glibenclamide or insulin. In overweight patients, the effect was seen with metformin with lower ***TC*** -0.42 (-0.65, -0.20) mmol/L, ***LDL*** -0.20 (-0.37, -0.02) mmol/L and ***TG*** -0.42 (-0.76, -0.09) mmol/L. These effects were similar but less pronounced by 6 years. **Conclusions :** The hypoglycaemic therapies studied had modest effects on plasma lipids, although chlorpropamide and, in overweight subjects, metformin reduced both ***TC*** and ***TG***. The chlorpropamide effect may have counterbalanced the increased blood pressure induced by this therapy. The metformin effect may have contributed to the reduced incidence of myocardial infarction.

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THE DIABETES ATHEROSCLEROSIS INTERVENTION STUDY (DAIS): BASELINE CHARACTERISTICS AND 2 YEAR LIPID RESULTS.
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DAIS is the first study designed *a priori* to test whether correcting dyslipoproteinemia in men and women (40-65yrs) with type 2 diabetes will alter coronary disease. It is a randomized double blind angiographic study using micronized fenofibrate (Feno) *vs* placebo (Plac) with 418 participants (305 men, 113 women; 250 Europeans, 168 Canadians). 218 had no previous clinical CAD, while 200 did. The mean baseline values were TG 2.42mM, total cholesterol (Tch) 5.57mM, calculated LDL-Ch (LDLc) 3.43mM, HDL-Ch 1.03mM, apoB 1.15g/L, BMI 28.8, HbA1c 7.53%, and fasting glucose 8.79mM. They did not differ between active and placebo groups. Though 1% of Canadians and 16% of Europeans were treated with oral agents plus insulin, their glycemic control did not differ. 15% were current smokers. Although 51% had been diagnosed with hypertension, the entry mean BP was 140/82. Coronary follow-up is still under way and can not be reported. After 2 yrs. 95.2% were still in the study and the mean adherence to medication was 96%, not differing between Feno and Plac groups. Thus we can provide the first report of the long term effects of fibric acid related drug on lipids in well controlled type 2 diabetes. The mean % changes in lipids were:

	TG	T-Ch	LDLc	HDL-Ch	apoB
Plac	2.1%	0.8%	+ 1.6%	+ 0.6%	+ 1.0%
Feno	-27.2%	-9.0%	-5.7%	+ 6.7%	-10.5%
p	0.0001	0.0001	0.0001	0.0001	0.0001

The hypoglycemic regimen did not influence the lipid response. There were no significant differences between Feno and Plac in the occurence of cancer, clinical gall bladder or liver disease, muscle symptoms, CNS problems , GI complaints or renal disease. Micronized fenofibrate is safe and effective in the long-term treatment of the typical lipoprotein abnormalities (TG↑, HDL ↓) in well controlled type 2 diabetes.

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PREVALENCE AND PROGRESSION OF DYSLIPIDAEMIA IN TYPE 2 DIABETES: THE FREMANTLE DIABETES STUDY.

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Aims: To investigate the prevalence and management of dyslipidaemia in type 2 diabetes in the community.

Materials and Methods: We analysed cross-sectional and prospective data from the Fremantle Diabetes Study (FDS), a study of care, control and complications in an urban multi-ethnic population. Each FDS patient has a full annual assessment and the results are reported to his/her usual doctor(s). Dyslipidaemic patients are defined as those on hypolipidaemic drugs or with a serum lipid profile justifying treatment under Australian Pharmaceutical Benefits Schedule criteria (serum total cholesterol >6.5 mmol/L, or >5.5 mmol/L with HDL-cholesterol <1.0 mmol/L).

Results: Complete data were available for four assessments in 676 patients, representing a mean±SD follow-up of 3.3±0.4 years. At baseline, 32.1% had dyslipidaemia and this increased to 47.3% at the 4th visit (P<0.001). Using multiple logistic regression, progression to dyslipidaemia was associated with body mass index (P=0.02), and inversely with HbA_{1c}, and diabetes duration (P=0.02). The percentage of dyslipidaemic patients treated with hypolipidaemic agents increased from 37.8% to 61.3% (P<0.001) during follow-up but the proportion on fibrate therapy fell from 36.6% to 16.8%. In those treated with a statin, the serum total cholesterol was ≤5.5 mmol/L in 62.3% and 62.7% at baseline and 4th visit, respectively (P>0.5). In both dyslipidaemic and normolipidaemic patients, body mass index and HbA_{1c} fell during follow-up while HDL-cholesterol increased (P<0.001) despite a progressive increase in the fasting plasma glucose.

Conclusions: 1. Diabetic dyslipidaemia is common in the community. 2. Its prevalence increases by 5% of patients/year. 3. The proportion of dyslipidaemic patients receiving treatment is increasing. 4. This increase is due to greater use of statins. 5. A significant proportion of dyslipidaemic patients may be under-treated. 6. Greater patient compliance with lifestyle measures and management, changes in management through FDS results, and/or management changes independent of the FDS may help lower body weight and contribute to improved serum HDL concentrations despite the progressive beta cell failure of type 2 diabetes.

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SIGNIFICANT IMPROVEMENT OF APOB-CONTAINING LIPOPROTEIN METABOLISM BY INSULIN TREATMENT, IN NIDDM PATIENTS.

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NIDDM patients exhibit multiple abnormalities of apolipoprotein B (apoB)-containing lipoproteins metabolism, which are likely to play an important role in the development of premature atherosclerosis, in these patients. When diabetes is poorly controlled, with oral antidiabetic agents, NIDDM patients usually receive insulin therapy. However, modification of apoB metabolism induced by insulin treatment, in NIDDM, are not precisely known. **Aims**: the aim of the study was to precise the effect of insulin treatment on apoB-containing lipoprotein metabolism, in NIDDM. **Materials and Methods**: We performed an *in vivo* stable isotope kinetic study, using L-[1-^{13}C] leucine, in 6 poorly controlled NIDDM patients, before and 2 months after the introduction of insulin therapy, and in 5 control subjects. **Results**: insulin treatment induced a decrease in VLDLapoB concentrations (121±42 vs 158±91 mg/l; p<0.05) due to an increased catabolism of VLDL towards IDL and LDL (0.20±0.08 vs 0.14± 0.07 pool/h; p<0.05). Insulin treatment induced an acceleration of IDLapoB turn-over, without changing its plasma level, by increasing both its production rate (22.6±9.2 vs 18.2±9.6 mg/l/h; p<0.05) and its catabolic rate towards LDL (0.34±0.22 vs 0.22±0.16 pool/h; p<0.05). Insulin treatment increased LDLapoB production rate (20.2±7.4 vs 16.9±7.7 mg/l/h; p<0.05) and LDLapoB catabolic rate (0.022±0.004 vs 0.018±0.004 pool/h; p<0.05), resulting in a constant LDLapoB plasma level. When these kinetic data were compared to controls, it appeared that LDLapoB catabolic rate was completely normalized, in NIDDM, after insulin treatment, when VLDLapoB and IDLapoB catabolic rates, although significantly improved by insulin therapy, were not totally normalized. **Conclusions**: insulin treatment, in NIDDM, induces profound metabolic modifications of apoB-containing lipoproteins, resulting in significant decrease of the intravascular residence time of VLDL, IDL and LDL particles (as a direct consequence of their increased catabolic rate). This is likely to reduce the atherosclerotic risk in such patients. Interestingly, LDLapoB catabolic rate, which is significantly decreased in poorly controlled NIDDM, is normalized by insulin therapy.

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AUTOANTIBODIES AGAINST OXIDIZED LDL AND ADVANCED GLYCATION END-PRODUCTS IN NIDDM WITH NEPHROPATHY

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Background. Oxidized LDL (oxLDL) plays an important role in the development of atherosclerosis. There is also evidence that advanced glycation endproducts (AGEs) are implicated in the pathogenesis of diabetic vascular complications. *The aim* of this study was to investigate the biological marker of *in vivo* LDL oxidation (autoantibodies against oxLDL) and serum AGE level in two age-matched groups of NIDDM patients (65 without microangiopathy and 27 with nephropathy), and in 25 normolipemic controls. Nephropathy was assessed on the basis of complete clinical examination. There was no difference in the glycemic control (HbA1c 7.9±2.5 *vs* 8.3±2.0), age (59.8±12 *vs* 61.1±9), diabetes duration (8.9±5.1 *vs* 9.9±4.9) and lipoprotein status (CH:6.2±1.3 *vs* 6.8±1.2, LDL-c:4.2±1.1 vs 4.3±0.9; TG:2.1±1.3 vs 2.6±1.7) between the two NIDDM groups. *Methods:* Serum IgG antibodies against oxLDL were determined by ELISA using malondialdehyde-derived LDL (MDA-LDL) as antigen. Competitive ELISA using polyclonal anti-AGE antibodies was used to measure AGEs. *Results:* The titer of autoantibodies against MDA-LDL was higher in both NIDDM groups vs controls (32.2±5.5 *vs* 20.1±4.8 AcU/ml, p<0.001). NIDDM patients with nephropathy showed higher oxLDLab titer than those free from microangiopathy (34.8±8.6 *vs* 28.1±7.3; p<0.05). A significant positive correlation was found between oxLDLab and urinary albumin/creatinine ratio (r=0.46, p<0.05). However, there was no correlation of oxLDLab with either standard lipid parameters or diabetes duration. OxLDLab was by 20% higher in NIDDM patients with coronary heart disease (n=5) than in NIDDM patients with nephropathy. A negative correlation was found between oxLDLab titer and metabolic control in both NIDDM groups (oxLDLab *vs* HbA1c, AGEs; r= -0.25, r= -3.3; p<0.001). Serum AGEs were significantly higher in NIDDM patients with nephropathy than in those free from microangiopathy (54.1±13 *vs* 47.1±14, p<0.05). A significant correlation was recorded between the parameters of early and advanced glycation (HbA1c *vs* AGEs p<0.0001). *In conclusion*, the results indicated the processes of oxidation and glycation to be more pronounced in nephropathy *in vivo* than in the age-matched NIDDM free from nephropathy, possibly contributing to the former group's susceptibility to accelerated vascular lesions.

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EFFICACY AND SAFETY OF ATORVASTATIN VERSUS SIMVASTATIN IN TYPE II DIABETES PATIENTS WITH CORONARY HEART DISEASE

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Aims: The Target Tangible study compared the effects of atorvastatin and simvastatin in a cohort of CHD patients in Germany. This subgroup analysis assessed the efficacy and safety of these agents in type II diabetes patients. **Materials and Methods**: Patients with LDL-C levels ≥130 mg/dL (3.4 mmol/L) entered a 6-week washout. Those meeting lipid entry criteria were randomized (2:1) to atorvastatin 10 mg or simvastatin 10 mg for 14 weeks. The dose was increased to 20 mg and 40 mg at weeks 5 and/or 10, respectively, if the target LDL-C of <100 mg/dL (2.6 mmol/L) was not reached. Primary endpoints were efficacy (responder rates) and safety (adverse events and laboratory measurements). Secondary endpoints were changes in lipid parameters including TGs. **Results**: 2856 patients met the entry criteria; 517 (18%) had diagnoses of type II diabetes. More diabetic patients (67%) reached their LDL-C goal than non-diabetics (61%) and significantly (p<0.001) more atorvastatin patients than simvastatin. Atorvastatin also achieved greater TG lowering.

Values on study completion	Diabetic		Non-diabetic	
	Atorva	Simva	Atorva	Simva
Patients achieving LDL-C goal	72 (%)	57 (%)	66 (%)	52 (%)
Median triglyceride lowering	−29 (%)	−15 (%)	−28 (%)	−21 (%)

There was no significant difference in adverse event rates between atorvastatin- and simvastatin-treated patients. Elevations of creatine kinase and liver enzymes >3× ULN occurred in 0.4% and 0.2%, respectively, of diabetics and 0.3% and 0.1% of non-diabetics with no significant difference in these parameters between atorvastatin- and simvastatin-treated patients. **Conclusions**: The benefits of statin treatment were significantly greater in diabetic than in non-diabetic patients. Atorvastatin was more effective than simvastatin in lowering LDL-C and TGs, and more type II diabetes patients reached LDL-C goals with atorvastatin than with simvastatin.

OP 10
Genetics of Type 2 Diabetes

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THE INSULIN GENE AND TYPE 2 DIABETES: EVIDENCE FOR LINKAGE AND ASSOCIATION MEDIATED EXCLUSIVELY BY PATERNALLY-DERIVED ALLELES.

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Aims: Variation at the VNTR (variable number tandem repeat) minisatellite 5' of the insulin gene (INS) is known to influence several phenotypes, including type 1 diabetes, polycystic ovarian syndrome (PCOS) and birthweight. In type 1 diabetes, the longer class III VNTR alleles are protective; in PCOS, they increase disease risk. Case-control studies have hinted at similar class III associations for type 2 diabetes, but results are inconsistent and may reflect confounding population stratification. The family-based association methods used in this study are not subject to this source of error and also allow detection of parent-of-origin effects (as seen at the VNTR in type 1 diabetes and PCOS). **Materials and Methods:** we studied 155 parent-offspring trios from the British Diabetic Association-Warren trios repository, each ascertained via a Europid proband with type 2 diabetes (age at diagnosis, 40(25-58)years, BMI 32.1(19.7-59.6)kgm-2, median(range)). Type 1 diabetes and MODY were excluded by clinical, biochemical (anti-GAD) and genetic means. VNTR class genotypes were inferred through analysis of the -23/HphI polymorphism: the two sites are in tight linkage disequilibrium in Caucasians. **Results:** Of 119 heterozygous parents, 65 (55%) transmitted class III vs 54 class I (p=0.16, binomial, one-sided). However, this overall result masked clear parent-of-origin effects. In the 54 informative maternal transmissions, there was no deviation from expectation (23(43%) class III vs 31 class I, p=0.28, two-sided). In contrast, fathers displayed marked excess class III transmission (34(69%) vs 15, p=0.003 vs 50% expectation, p=0.003 vs maternal transmission). **Conclusions:** Using family-based association methods, we have provided the strongest evidence yet that variation at the INS-VNTR locus is a major influence on type 2 diabetes susceptibility. For the first time, we have demonstrated that this effect is mediated exclusively by the paternally-derived VNTR allele, raising the possibility that imprinted genes play a crucial role in the pathogenesis of type 2 diabetes.

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CLONING OF THE 5' FLANKING REGION OF THE MOUSE INSULIN RECEPTOR SUBSTRATE -3 GENE.

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Aims: Aim of this study has been to clone and analyse the 5' flanking sequence of the recently cloned the mouse Insulin Receptor Substrate-3 (mIRS-3) gene in order to identify the mechanisms by which its expression is regulated. Insulin Receptor Substrate (IRS) proteins play an essential role in insulin-mediated mitogenic and metabolic signaling. IRS-3 gene is highly expressed in the first part, and almost undetectable in the last part of the mouse embryonic life. **Materials and methods**: Screening of mouse P1 genomic DNA library was conducted using a 5' portion of the mIRS-3 coding sequence as a probe. One P1 clone has been isolated, the DNA digested with Hind III, and subcloned into a pBluescript vector. A subclone containing the mIRS-3 entire gene and a 1,800 bp fragment at the 5' flanking region of the gene was isolated by colony hybridization. The nucleotide sequence was determined by automatic sequencing and compared to the promoter sequences in the eucharyotic promoter database. In order to identify the transcription starting sites, total RNA was isolated from mouse liver, and used as template for a labeled-primer elongation experiment. **Results**: The 5' region of the mIRS-3 gene lacks typical CAAT and TATA boxes but contains several potential Sp1 binding sites, consistent with a housekeeping gene. Two main transcription starting sites were identified at nucleotide position -361 and -179 respectively. Significant regions of identity with other mouse gene promoters, such as Hypoxantine-Phospho-Ribosyl-Transferase (nt -1564 to -1548 and -1403 to -1319 respectively), Interferon alpha 5 (nt -1327 to -1277) and Ki-Ras (nt -1341 to -1324) were identified. Within these high homology regions, potential RxR and glucocorticoid receptors interacting sites were present. The sequence homology as well as the presence of similar potential transcription regulating sites, suggest the possibility that the expression of mIRS-3 gene might be regulated by at least some of the mechanisms acting for these genes. **Conclusions:** We have cloned and sequenced the 5' flanking region the mIRS-3 gene. The complete functional analysis of the mIRS-3 promoter region, and the identification of the factors that modulate its expression during both the embryonic development and adult life, will contribute to elucidate the specific role of this gene in mediating the mitogenic and metabolic effects of insulin.

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The Tumour Necrosis Factor-Alpha –308 Promoter Polymorphism Is Associated With Increased Insulin Sensitivity In Caucasian Australian Females.

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The TNF-alpha gene has been highlighted as a candidate gene that may be involved in the development of insulin resistance in obese individuals. Several investigators have identified sequence variation within the TNF-alpha gene and a number of polymorphisms have been variably associated with insulin resistance. **Aim:** In this study we investigated the *Nco1* polymorphism at position –308 of the TNF alpha promoter region. This polymorphism has previously been associated with increased transcription of the TNF alpha gene *in vitro* and we examined associations between the presence of this polymorphism and obesity and type 2 diabetes in humans. **Materials and methods:** Study subjects included 311 males from Nauru (mean BMI:36), a population with a particularly high prevalence of obesity and type 2 diabetes. We looked for associations between this polymorphism and diabetes- and obesity-related phenotypic markers. In addition, we examined the -308 polymorphism in 483 Australian women (mean BMI:27), a population with relatively low rates of obesity and diabetes. Diabetes status was defined using the ADA classification. **Results:** The –308 polymorphism is a G to A base change and in the Nauruan population there were no subjects with the A/A genotype and the A allele was present at a frequency of only 0.01. In contrast, in the Australian population, the frequency of the A allele was 0.21. Interestingly, in the Australian population, women with the A/A genotype (n=52) had significantly lower fasting insulin concentrations (7.98μU/ml) compared to subjects with the G/A (9.62μU/ml) or G/G (9.52μU/ml) genotypes, adjusted for age and percent body fat ($p<0.05$). Of interest also was the finding that in diabetic women, who were already hyperinsulinemic, the A/A genotype (n=8) was associated with a lower BMI, smaller waist circumference and markedly decreased circulating leptin concentrations ($p<0.05$). **Conclusions:** These findings suggest that sequence changes in the TNF alpha promoter may be associated with increased insulin sensitivity in non-diabetic Caucasian women. In addition, the findings in diabetic women implicate a role for TNF alpha in body fat accumulation and distribution which warrants further investigation.

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K121Q POLYMORPHISM IN EXON 4 OF PC-1 GENE IS STRONGLY ASSOCIATED WITH INSULIN RESISTANCE

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Aim and Methods: we first identified by Single Strand Conformation Polymorphism and sequencing a polymorphic single base change (K121Q) in the PC-1 gene exon 4. We then investigated the association between this PC-1 gene alteration and insulin resistance in 121 unrelated healthy, normal glucose tolerant (by OGTT) subjects and 135 patients with type 2 diabetes. Insulin receptor tyrosine kinase activity (IRTK) was also studied in cultured skin fibroblasts from 10 healthy subjects, 5 with and 5 without the Q allele. Results: As compared to 80 KK subject, Q allele carriers (n=41, 39 KQ and 2 QQ) showed higher glucose and insulin levels during OGTT ($p<0.001$ by 2-way ANOVA) and insulin resistance as indicated by euglicaemic clamp (M value=5.25±1.38 mg/Kg/min, n=24, vs. 6.30±1.39, n=49, p=0.005). When subjects were subdivided in tertiles according to insulin sensitivity, Q allele frequency was lower in individuals (n=81) with high or intermediate insulin sensitivity (11.7%) than in subjects (n=40) who were insulin resistant (27.5%, p=0.003) and in type 2 diabetic patients (n=135, 20.7%, p=0.02). Insulin stimulation of IRTK activity was reduced ($p<0.01$) in cultured skin fibroblasts from 5 healthy KQ vs. 5 matched KK subjects. Conclusions: PC-1 gene polymorphism K121Q is strongly associated with insulin resistance in both healthy subjects and in type 2 diabetic patients. Our data raise the possibility that PC-1 genotyping could identify individuals who are at risk of developing insulin resistance, a condition which predisposes to type 2 diabetes and coronary heart disease.

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LEPTIN RECEPTOR GENE POLYMORPHISMS ARE ASSOCIATED WITH INSULIN IN OBESE DIABETIC WOMEN.

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Aim of the study : Serum leptin and insulin levels are well correlated, and leptin administration to rodents enhances insulin sensitivity and glucose utilisation. Leptin receptors are present on β-cells, as well as on muscle and fat cells. Leptin inhibits especially the glucose-stimulated insulin secretion from pancreatic cells. Based on these data, we hypothesized that the leptin receptor (LEPR) could play a role in regulation of insulin levels after an oral glucose load. In this study, the possible effect of polymorphisms in the LEPR gene was investigated. Methods : Three LEPR polymorphisms *Lys109Arg, Gln223Arg* and *Lys656Asn* were typed on genomic DNA. A total of 372 overweight and obese women (BMI>25), aged 18-60, were evaluated for diabetic state with an OGTT according to the WHO-criteria. Results : Out of these 372 women, 280 showed normal glucose tolerance, while 92 showed impaired glucose tolerance (IGT) or type 2 diabetes. Statistical analyses were performed by carrier status with a GLM-procedure, in two age groups, 18-45 and 45-60 years, after adjusting the data for age and fat mass. In women aged 45 to 60 with IGT or diabetes (n=32), significant associations were found with *Lys109Arg* for fasting insulin and 2h insulin response on OGTT (both p=0.04). In the same group, trends were seen for fasting insulin with the two other polymorphisms, for 2h insulin with *Gln223Arg*, and for the area under the curve with *Lys109Arg* and *Gln223Arg*. In contrast, in younger women, no association could be found. Conclusion : These data seem to indicate that LEPR polymorphisms are associated with insulin response in older women with impaired glucose homeostasis.

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SYNERGISTIC EFFECT OF POLYMORPHISMS IN UNCOUPLING PROTEIN 1 AND β_3-ADRENERGIC RECEPTOR GENES ON LONG-TERM BODY WEIGHT CHANGE IN FINNISH TYPE 2 DIABETIC AND NON-DIABETIC CONTROL SUBJECTS

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Aims: To investigate the independent and combined effects of the Trp64Arg polymorphism of the β_3-adrenergic receptor gene and the (-3826) A→G polymorphism of the uncoupling protein 1 gene on body weight change in Type 2 diabetic and non-diabetic control subjects in a prospectively conducted 10-year follow-up study. **Subjects and methods:** 70 newly diagnosed, middle-aged Type 2 diabetic patients and 123 non-diabetic control subjects from eastern Finland. Genotypes were assessed by polymerase chain reaction followed by enzymatic digestion. **Results:** There were no significant differences in the frequencies of the two polymorphisms between diabetic and control subjects. The polymorphisms were not cross-sectionally or longitudinally associated with body weight or BMI in diabetic or control subjects. When the diabetic and control subjects were analysed together after adjusting for age and sex, glucose tolerance status, and antihypertensive and hypoglycemic drug treatment, the change in the mean body weight was significantly greater among the subjects with both polymorphisms (n=11) than among those with no polymorphisms (n=103), (change in weight 6.5±2.5% vs -0.2± 0.8%, p=0.036, and change in BMI 8.5±2.6% vs 2.0±0.8%, p=0.060), mean±SEM). **Conclusions:** The Trp64Arg and A→G polymorphisms of the β_3-adrenergic receptor and uncoupling protein 1 genes are not major independent factors for body weight gain and obesity in Finnish subjects. However, in the present study the simultaneous existence of the two polymorphisms was associated with a tendency to gain weight suggesting a synergistic effect of these polymorphisms on body weight gain.

OP 11
Diabetes and Pregnancy

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INSULIN AND GROWTH HORMONE SECRETION IN MODERATELY OVERWEIGHT NEWBORNS DURING EARLY NEONATAL PERIOD.

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The aim of the research was to study the peculiarities of insulin and growth hormone (GH) secretion in healthy newborns from nondiabetic mothers (38-40 weeks of gestation) depending on their birth weight. Materials and methods: IRI, C-peptide (C-p), GH levels (ng/ml) and C-p/IRI molar ratio were measured in neonatal blood serum of umbilical vein immediately after birth and cranial vein on the 7-th day after the delivery (103 normalweight 2500,0<NW<4000,0; 154 overweight OW≤4350,0). Morphometric investigation of islet tissue of pancreases obtained from 18 NW and 22 OW foetuses, died from birth injury was also performed. Results: OW group had significantly (P<0.05) increased IRI, C-p, C-p/IRI levels (0.42±0.09; 2.34±0.08; 5.6±0.07), respectively and decreased GH level at birth (9.7±0.8, P<0.01), compared to NW (0.30±0.02; 1.01±0.07; 3.4±0.3; 26.2±1.3, respectively). OW foetuses had 2.5-fold increased (P<0.02) percentage of endocrine tissue in caudal part of the pancreas, 2.6 times greater(P<0.01) content of ß-cells inside the islets. A and D-cells content did not differ between the groups. At the 7-th day after the delivery and beginning of breast feeding infants' birth weigh did not change significantly (P>0.5). At the same time no changes of IRI level in both groups and of C-p, C-p/IRI ratio in OW-s was found, whereas the latter indices enhanced 3-and 4-fold (p<0.01), respetively in NW infants. During this period the decrement of GH level was observed in all neonates but its concentration remained significantly lower in OW-s compared to NW-s (7.1±0.7; 13.5±0.85, respectively, P<0.001). Conclusion: Moderately overweight infants from nondiabetic mothers revealed ß-cell hyperplasia in conjunction with insulin hypersecretion at birth and decreased ß-cell reactivity to the breast feeding at the 7-th day after delivery. Relatively low level of GH in OW newborns preserved during the first week of extrauterine life. These changes may be considered as predictors of future development of obesity and insulin resistance.

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DOES MATERNAL DIABETES IMPAIR LONG CHAIN ESSENTIAL FATTY ACID SYNTESIS IN MOTHER FETUS AND BREAST MILK.

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Aims: Adequate supplies of Arachidonic (AA) and Docosahexaenoic acid (DHA) are vital to fetal development. Diabetes impairs the delta-6 desaturase enzyme required for AA and DHA synthesis. To determine whether dietary intake or the presence of diabetes altered the plasma long chain essential fatty acid concentrations, we examined dietary intake, maternal & cord plasma & mature breast milk levels. **Method:** Mothers 13 control (C), 10 gestational (GDM), 9 established diabetics (EDM) kept 4 day mid trimester food diaries. Third trimester maternal & cord blood & breast milk was collected. The plasma fatty acids were separated by GLC. **Results:** The total intake of n-6 & n-3 fatty acids was similar between the 3 groups. Maternal & cord plasma choline phosphoglyceride (CPG) total saturated fatty acids & total n-6 metabolites were similar, but total n-3 metabolites were lower in the cord plasma of the EDMs compared with the C & GDMs (5.0±0.49%*, 7.1±0.49%* 6.5±0.38% respectively). Cord plasma CPG α linolinic acid was universally low; in contrast the CPG DHA% was over 50 fold greater but significantly lower in the EDM compared with C & GDM .(4.2±0.45%*,6.0±0.39%*,5.4±0.33% respectively). GDM & EDM Breast milk contained significantly more total saturated fatty acids than the C milk. Milk DHA levels were marginally lower in EDMs compared with the C & GDMs (0.34±0.04%* 0.46±0.05%* 0.48±0.07% respectively). **Conclusions:** The control & EDM women eat similar quantities of fats but their fatty acid plasma choline phosphoglyceride composition was significantly different; the EDM % total saturated fat was higher & the total n-3% was lower. The same trend was observed in the cord plasma & breast milk. Synthesis of DHA appears to be reduced in the EDM women. A similar pattern is observed in their breast milk & the cord plasma. (* significantly different to corresponding control values p< 0.05. ± = SEM)

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MALFORMATIONS IN NEURAL CREST DERIVED FETAL ORGANS AND EFFECTS OF MATERNAL VITAMIN E TREATMENT IN DIABETIC RAT PREGNANCY.

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Children of diabetic mothers show increased rate of cardiac malformations, in particular of the out-flow tract, which may be a consequence of impaired development of the cranial neural crest cells (NCC). **Aim:** to study the development of NCC derived fetal organs in a rat model of diabetic pregnancy, with or without maternal antioxidant treatment. **Materials and methods:** rats of a malformation-prone strain were given streptozotocin-induced diabetes prior to pregnancy, which was interrupted on day 16 or 20. **Results:** fetuses of the diabetic rats showed low set external ears, severely malformed Meckel's cartilage, small thyroid and thymus and absence of parathyroid glands. Cardiac anomalies included rightward displacement of the aorta, double outlet right ventricle (DORV), persistent truncus arteriosus (PTA) combined with ventricular septal defects due to a malaligned outlet septum. The malformations in the outflow tract covered abnormalities of the great arteries; right sided aortic arch/descending aorta, and double aortic arches. Maternal dietary treatment with 2 % vitamin E markedly reduced the severity of the malformations. **Conclusions:** The phenotypic appearance of these defects is strikingly similar to the DiGeorge anomaly in humans, which has been found in children of diabetic mothers, together with an over-representation of PTA and DORV. The teratogenic mechanisms may be similar, and accessible for study.

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CHANGED PKC ACTIVITY IN EMBRYOS EXPOSED TO HIGH GLUCOSE MAY CAUSE DYSMORPHOGENESIS

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Glucose-induced teratogenesis has been associated with disturbed inositol metabolism in the embryo which may alter protein kinase C (PKC) activity. **Aim:** to study the effect on embryonic development *in vitro* of changed PKC activity. **Materials and Methods:** we exposed *in vitro* embryos to 30 mM glucose (30G), to 500 μM or 750 μM of *scyllo*-inositol (500SI and 750SI, an inhibitor of inositol uptake), and to 1-5 μM of the PKC-inhibitor GF-109200X. **Results:** 30G, 500SI and 750SI embryos had lower somite number (16.3, 21.5 and 19.5) and higher malformation score (MS; 9.4, 6.4 and 6.9) than control embryos cultured in 10 mM glucose (10G) (somites: 28.7, MS: 0.1). Adding 1600 μM inositol to the 30G or 750SI culture medium partly corrected these embryos (somites: 23.6-23.9, MS: 3.7-4.2), and completely normalized the 500SI embryo development (somites: 28.6, MS: 0.6). 1-5 μM PKC inhibitor yielded a dose-dependent decrease in somite number (down to 12.5) and an increase in MS in 10G (MS up to 9.5-10.0), and no normalization of the changed somite number and malformation score at 30G, despite addition of the inhibitor. **Conclusions:** high glucose concentration suppresses the embryonic metabolism of inositol yielding decreased PKC activity, which disturbs embryonic development. Blocking this teratological pathway may diminish embryopathy in diabetic pregnancy.

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CORD BLOOD INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN (IGFBP-1) IN THE CONTROL OF FETAL GROWTH IN DIABETIC PREGNANCY

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The size of human infant at birth depends on several factors: genetic constitution, nutritional status and pathological conditions(maternal diabetes, pre-eclampsia or infections). Despite extensive investigation, as yet, no specific endocrine mechanisms which would play an essential role in fetal growth have been identified. Recent attention has been focused on the role of growth factors (IGFs) and their binding proteins (IGFBPs) in the control of fetal growth. The aim of the study was to evaluate, if cord blood IGFBP-1 plays any role in the control of fetal growth. The study group consisted of 81 pregnant women with pregestational and gestational diabetes. Their glycemia was estimated by daily self-monitoring, by monthly performed diurnal glucose profiles as well as by measurements of HbA_{1C} concentration. Cord blood insulin and IGFBP-1 levels were analysed by MEIA and ELISA technology. In the respective trimesters, in the analysed group of pregnancies the mean glycemia level was as follows: 114 mg/dl, 100 mg/dl and 98 mg/dl. The HbA_{1C} concentrations were 7,7%, 7,5% and 7,4% respectively (normal value 3,8%-6,3%). Eleven (13,6%) newborns achieved body weight (BW) exceeding the 90 percentile, 25 (31%) presented with BW below the 25 percentile. There was no correlation between maternal glycemia, cord blood insulin levels and newborns' BW, but cord blood IGFBP-1 concentration correlated with newborns BW ($p.<0,001$). The concentration of IGFBP-1 was significantly lower in the group of newborns with hypotrophy than with in those with overweight. Conclusion: In the well controlled diabetic pregnancy IGBP-1 of fetal origin may play an essential role in the regulation of fetal growth.

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CHANGES IN THE HAEMOSTATIC SYSTEM IN PREGNANT WOMEN WITH DIABETES MELLITUS TYPE 2 IN THE PERINATAL PERIOD.

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In the perinatal period the fluctuations of some haemostatic parameters were observed. In patients with type 2 diabetes the disturbances of the haemostatic system were found. The aim of our study was to evaluate some haemostatic parameters during the perinatal period in pregnant women with type 2 diabetes (DMP). The study was carried out on 10 DMP (mean aged 36,5 ± 5,5) with a good metabolic control (HbA_{1c} 5,2 ± 1,1%). The control group (CG) contained 24 healthy pregnant women (mean aged 25,6 ± 4,5). The blood was taken between 36-38 weeks of pregnancy (the III-rd trimester - T3), two hours after delivery of placenta (AD) and after puerperium (AP). The following haemostatic parameters were estimated: platelet count (PLT), activity of antythrombin III (AT III) and plasminogen activator inhibitor type 1 (PAI-1) as well as the concentration of tissue plasminogen activator antigen (tPA:Ag), fibrinogen (F) and fibrinogen/fibrin degradation products (FDP). The PLT in DMP in comparison to CG was significantly lower AD ($p<0,05$). The tPA:Ag concentration and PAI-1 activity were significantly higher AD ($p<0,05$ for tPA:Ag and $p<0,01$ for PAI-1) and AP ($p<0,05$ for tPA:Ag and $p<0,01$ for PAI-1) in DMP in comparison to CG. The FDP concentration in DMP was significantly lower AD and AP in comparison to CG ($p<0,01$ and $p<0,05$ respectively). We also compared values of these parameters in DMP in 3 periods (table below, M±SD).

Period	PLT (G/l)	AT III (%)	tPA:Ag(ng/ml)	PAI-1(IU/ml)	F (g/l)	FDP(μg/ml)
T3	161±47,2	111±27,3	14,2±13,5	22,2±8,2	3,7±1,5	5,6±5,8
AD	119±19,5•	124±30,6	19,1±9,3	14,0±2,8•	3,0±1,5	5,7±4,5
AP	157±23,5•	121±17,3	12,6±5,0	13,6±4,0•	2,3±0,3•	3,3±3,5

•$p<0,05$ •$p<0,01$

The PLT significantly decreased AD in comparison to T3 and then significantly increased AP. The significant decrease of PAI-1 activity was observed AD and AP in comparison to T3. The significant decrease of F concentration was found AP in comparison to T3. Conclusion: the fibrinolytic activity in pregnant women with type 2 diabetes in the perinatal period is decreased in comparison to healthy pregnant women. It could be the risk of thrombotic complications in these group of patients.

OP 12
Endothelium

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ACTIVATION OF THE VASCULAR ENDOTHELIUM AND Na^+-Li^+-COUNTERTRANSPORT IN TYPE 2 DIABETES

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In type 2 diabetes, we previously found that albumin excretion rate (AER) was correlated with cardiovascular risk factors. Cardiovascular risk factors and diabetic nephropathy have shown associations with the elevated sodium-lithium countertransport (SLC) activity. To investigate the relationships between activation of the vascular endothelium, SLC and AER, plasma levels of the adhesion molecules sE-selectin, sICAM-1, sVCAM-1, von Willebrand factor (vWF), and SLC were measured in a cohort of 75 type 2 diabetic patients, mean age 60 ± 11 years, diabetes duration 12 ± 8 years. Patients were classified into normoalbuminuric (AER < 20 µg/min, n= 32) and microalbuminuric (AER 20 - 200 µg/min, n= 33). Adhesion molecules were determined by enzyme immunoasssay. SLC activity was measured as Na^+-stimulated Li^+ efflux from Li^+-loaded erythrocytes; maximal rate of turnover (V_{max}) was calculated using the Eadie-Hofstee plot. Compared with normoalbuminuric patients, microalbuminuric patients had higer levels of sE-selectin (69.4 ± 32.2 vs. 53.5 ± 19.9 ng/ml, p= 0.013), being related to both SLC activity (r= 0.459, p= 0.000) and to V_{max} (0.441, p= 0.000). There was no direct association between sE-selectin and AER. Multiple regression analysis showed V_{max} (r= 0.384, p= 0.011) and systolic blood pressure (p= 0.017) to be determinants of AER. Levels of sICAM-1 (271.1 ± 76.6 vs. 279.7 ± 74.2 ng/ml) and sVCAM-1 (705.3 ± 224.7 vs. 679.8 ± 188.4 ng/ml) were neither significantly different between the patient groups nor were they related to sE-selectin, vWF or AER. The increased levels of sE-selectin and their association with SLC in type 2 diabetes with microalbuminuria suggests that these factors may be related to the activation of vascular endothelium and to the risk of cardiovascular complications.

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EARLY MARKERS OF ENDOTHELIAL DYSFUNCTION IN TYPE 1 DIABETES MELLITUS

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Aims: To evaluate selected biochemical variables as markers of early vascular changes in Type 1 diabetes. **Patients and Methods:** We examined 33 Type 1 diabetic patients (18 men and 15 women, mean age 39±8 yrs) in a 6 year follow-up study. The whole cohort was separated into patients who did not develope diabetic retinopathy (Group A, n=10), who developed or worsened diabetic retinopathy during 6 years (Group B, n=12) and who had persisted retinopathy (Group C, n=11) since the beginning of observation. The ophthalmological findings were confirmed by fluorescence angiography. Control group consisted of 20 healthy persons of comparable age and body mass index. Plasma tissue plasminogen activator (tPA) and its inhibitor (PAI-1), plasma free N-terminal 30-kD fibronectin domain (30-kD FN) as well as albumine: creatinine and glycosaminoglycan:creatinine ratios (GAG) in urine were determined at the beginning of the follow-up. **Results:** Their results are expressed as means and 2SD range or means±SD in the Table.

	Group A	Group B	Group C	Controls
30 kD FN (µg/l)	772±162	906±216*	1040±194**	645±142
tPA (ng/ml)	4.4 (1.7-11.7)	6.6** (3.0-14.5)	7.4** (4.5-12.2)	4.9 (2.8-8.7)
PAI-1 (ng/ml)	20.0 (4-100)	46.3** (13-160)	23.4 (4-135)	45 (21-99)
Albuminuria (g/mol creat.)	0.44 (0.12-1.62)	0.63 (0.1-4.27)	1.06** (0.37-3.04)	0.45 (0.17-1.14)
GAG (g/mol creat.)	2.4 (0.7-8.6)	4.4** (1.2-16.1)	3.9* (1.6-9.2)	2.0 (0.8-5.1)

Statistical significance as compared to Group A: *p<0.05, **p<0.01.

Significantly higher plasma tPA, PAI-1 and free 30-kD fibronectin domain and GAG in urine were found in diabetic patients before development or worsening of retinopathy (Group B) as compared to those without changes. **Conclusions:** Selected biochemical variables may be used as markers of endothelial dysfunction in diabetes.

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INCREASED PROCARBOXYPEPTIDASE U LEVELS IN TYPE II DIABETES.

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Aims: Diabetics, type II diabetics in particular, are characterized by an increased cardiovascular risk. Among different factors triggering this risk, alterations in haemostasis and fibrinolysis such as increased levels of fibrinogen and PAI-1 activity are well known in diabetics. Carboxypeptidase U (EC3.4.17.20:CPU,TAFI) is a recently discovered basic carboxy-peptidase which is formed in plasma from its precursor proCPU during the processes of coagulation and fibrinolysis. The most important physiological function is believed to be a retardation of clot lysis. Materials and Methods: In order to evaluate whether proCPU levels are elevated in diabetes, we measured, in this preliminary study, proCPU levels in type II male and female diabetics (n=136) and compared them to an age (40-70y)-matched control group (n=272). Results: Mean proCPU levels reached 1103±170 U/L (mean±SD) in diabetics (697-1687 U/L) whereas in non-diabetics a significantly (p<0.001) lower mean CPU level (985±159 U/L) was found (440-1456 U/L). Both for men and women separately, higher proCPU levels were found in diabetic compared to non-diabetic subjects (p<0.001). In diabetics a limited relationship (r=0.20;p=0.028) existed between proCPU and HbA1C, without any relationship with fasting glucose levels. Conclusions: ProCPU, a recent described parameter related to coagulation seems to be clearly increased in male and female type II diabetics. Diabetes metabolic control may have a modest influence on their serum levels. Further studies are needed to establish other determinants.

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BLUNTED RESPONSE OF PLASMA ENDOTHELIN-1 AND ACTIVE RENIN LEVELS TO POSTURAL CHANGES IN PATIENTS WITH UNCOMPLICATED TYPE-II DIABETES

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Aim: The present study was performed in patients with uncomplicated type-II diabetes to investigate plasma endothelin-1 response to postural changes and its relationship to corresponding plasma active renin levels. Materials and Methods: Twenty-two individuals (7 female and 15 male) aged 49±4 years with BMI 28±3 kg/m with type-II diabetes and 12 healthy subjects aged 50±3 years with BMI27±2kg/m. Patients with diabetic retinopathy, nephropathy or cardiovascular disease were excluded from the study. Blood samples were drawn at baseline 0, 5 and 10 minutes during a head-up tilt test and 30 minutes and 1 hour of standing. Endothelin-1 was determined by competitive radioimmunoassay (Peninsula Lab.Inc,USA) and plasma active renin was measured by immonoradiometric assay (Nichols Institute, USA). All measurements were done in duplicate and the average was used for the determination of means±SD. For the statistical evaluation of the results, the one-way analysis of variance was used. Results: Basal endothelin-1 levels did not differ significantly between the two groups and did not show any significant difference during the head-up tilt test in either group. However, after 30 minutes of standing, they displayed a significant increase compared to the basal in both patients (P<0.001,F:8.9,basal M±SD: 11.2±4.3pg/ml v.s 30min M±SD:26.1±19.4 pg/ml) and controls (P<0.001, F:409.8, basalM±SD:7.7±1.6pg/ml v.s 30min M±SD:28.2±0.6pg/ml). Interestingly, the values of endothelin-1 after 1 hour of standing did not change significantly as compared to the 30min values. In contrast a significant increase was observed in the healthy group (p<0.05,F:24.7,30min of standing M±SD: 28.2±0.6pg/ml v.s 1 hour of standing M±SD:32.7±1.9pg/ml). Plasma active renin in diabetics exhibit higher basal values:27.54±20.9µU/ml compare to the controls:9.78±3.1µU/ml. Nevertheless, it showed a similar profile during the head-up tilt test. A significant increase was observed after 30min of standing compare to the basal values (p<0.002,F:3.29, basal M±SD:27.54±20.9µU/ml v.s 30 min of standing M±SD:46.6±42.1µU/ml). In line with endothelin-1 pattern, plasma active renin in diabetics showed a blunted response during the 30min to 1 hour of standing although a significant increase was observed in healthy subjects (p<0.004,F:5.73,30 min ±SD:26.9±6.6µU/ml v.s 1 hourM±SD:36.26±5.6µU/ml-control group). Conclusions: These results indicate that patients with uncomplicated type-II diabetes exhibit a higher basal plasma active renin levels and a blunted response of both plasma endothelin-1 and active renin between 30min and 1 hour of standing compared to the healthy individuals.

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NEGATIVE CORRELATION OF HIGH SENSITIVE CRP WITH INSULIN SENSITIVITY AND ENDOTHELIAL FUNCTION IN FIRST DEGREE RELATIVES OF TYPE2 DIABETICS

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Aims: Endothelial dysfunction is an early abnormality in the development of atherosclerosis. Insulin resistance is discussed to be an independent risk factor for atherosclerosis. In addition Atherosclerosis is thought to be a chronic inflammatory disease. We therefore measured biochemical and molecular markers for inflammation in vivo and looked for correlations with insulin resistance and endothelial dysfunction. Patients and Methods: We investigated 49 normotensive and normoglycemic first degree relatives of subjects with type 2 diabetes (22m, 27f, age 32.2±8.4 y, BMI 24.7±4.6 kg/m^2, HbA1c 5.1±0.4%, 24h blood pressure 120.5/72.3±9.8/7.0 mm Hg). Flow associated vasodilatation (FAD%) as a parameter for endothelial function was measured with a high resolution ultrasound system (13.0 MHz). Insulin sensitivity expressed as metabolic clearance rate (MCR) was derived from an euglycemic clamp. Soluble intercellular adhesion molecule 1 (ICAM1) was measured by chromogenic substrate test, c reactive protein (CRP) by turbidimetric and high sensitive CRP (CRPhs) by nephelometric method. Values are given as means ± SD, Spearman correlation test. Results: FAD% = 5.3±2.6%, MCR = 7.4±3.8 ml/kg min^{-1}, ICAM1=141.6±29.2 ng/ml, CRP= 0.16±0.11 mg/dl, CRPhs = 1.0±0.9 mg/l. FAD% positively correlates with MCR (R = 0.45, P = 0.0013). There is a tendency for a negative correlation between ICAM1 and MCR (R = -0.25, P = 0.085), CRP does not correlate with MCR (R = 0.05, P = 0.73). Whereas, there is a significant negative correlation with CRPhs and MCR (R = -0.35, P = 0.01). Conclusions: Our data indicate that the measurement of CRPhs and possibly ICAM1 might be useful markers to identify people with incipient atherosclerosis and therefore increased cardiovascular risk.

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HEPATOCYTE GROWTH FACTOR INCREASES IN TYPE 2 DIABETIC PATIENTS WITH INSULIN RESISTANCE.

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Aims : Hepatocyte growth factor (HGF) has been reported as an important cytokine for vasculer remodeling. We investigated the possible involvement of HGF with insulin resistance and macroangiopathy in type 2 diabetic patients. **Mathelials and Methods** : We recruited 29 non-diabetic subjects (N) and 78 type 2 diabetic patients (DM) without diabetic microangiopathy, other disease, their past history of atherosclerotic disease nor alcoholic habit for this study. We measured serum HGF level (ng/ml) in the subjects with ELISA (hHGF kit : Ootsuka, Japan). We estimated insulin sensitivity (S.I.) using minimal model analysis and measured intimal medial thickness (IMT) at carotid artery with ultrasonography in type 2 DM. **Results** : Serum HGF levels were significantly correrated with body mass index (N : r=0.6484, p<0.001, DM : r=0.439, p<0.001) and S.I. (N: r=-0.284, p<0.01, DM: r=-0.515, p<0.001) in both groups. Serum HGF levels in 33 non-obese type 2 DM with insulin resistance (S.I.< 2.0×10^{-4}/min · μU/ml) were significantly higher than those in 29 non-obese type 2 DM without insulin resistance (0.231±0.056 vs 0.186±0.056, mean±SD, p<0.01). Moreover, serum HGF levels tended to incresse according to increment of IMT in type 2 DM. However, there was no correlation between serum HGF level and other clinical factors, such as, gender, age, blood pressure, serum lipids, fasting plasma gluose, HbA1c, fasting serum insulin and C-peptide. **Conclusions** : Serum HGF level increases in insulin resistant state with or without obesity in type 2 DM and maight be concerned with the development of atherosclerosis.

OP 13

Kinase Signalling Cascades and Insulin Secretion

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The Role of Phosphatidylinositol 3-Kinase in Glucose-Stimulated Insulin Gene Transcription

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Glucose stimulates insulin expression primarily through activation of the homeodomain transcription factor PDX1. This occurs via the triggering of the stress activated protein kinase 2 (SAPK2/p38) cascade, which leads to phosphorylation and activation of a cytoplasmic form of PDX1, which then translocates to the nucleus where it binds to its recognition sequences within the insulin promoter and stimulates gene transcription. The effects of glucose can be mimicked by the stress-inducing agent sodium arsenite. Glucose, but not arsenite, activation of SAPK2 and PDX1 is inhibited by wortmannin and LY294002 at concentrations that are known to inhibit phosphatidylinositol 3-kinase (PtdIns 3-kinase). However, these inhibitors affect other enzymes. Therefore to characterise further the role of PtdIns 3-kinase in the regulation of PDX1 DNA-binding and insulin promoter activity, we transfected MIN-6 β cells with a dominant negative form of PtdIns 3-kinase (Δp85). In untransfected cells glucose stimulated PDX1 DNA-binding activity, as measured by electrophoretic mobility shift assay, almost 2.5 fold (n=8). This effect was completely blocked in cells overexpressing Δp85 PtdIns 3-kinase. To study the effect on the insulin promoter, MIN-6 cells were transfected with the plasmid pGLLUC-200 which contains a fragment (-65 to -265) of the human insulin promoter upstream of the firefly luciferase gene. Glucose stimulated the activity of the construct 2.5 fold (7004 ± 3651 in low glucose, n=4 and 17538 ± 6226 in high glucose, n=4) at 16 mM compared with 0.5 mM. In cells co-transfected with pGLLUC-200 and Δp85 PtdIns 3-kinase the stimulatory effect of glucose was 1.5 fold (4600 ± 635 in low glucose, n=4 and 7507 ± 2962 in high glucose, n=4). These results provide strong evidence that PtdIns 3-kinase is involved in the pathway whereby glucose activates PDX DNA-binding and insulin promoter activity.

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POSSIBLE INVOLVEMENT OF ATYPICAL PROTEIN KINASE C IN GLUCOSE-INDUCED PHOSPHORYLATION AND ACTIVATION OF PDX-1.

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Aim: We previously reported that both DNA-binding activity and transcriptional activity of pancreatic and duodenal homeobox gene-1 (PDX-1) were increased with 20 mM glucose compared with 2 mM glucose, and involvement of PDX-1 phosphorylation in this event. In this study, we tried to identify the protein kinase which phosphorylate PDX-1 and the effect of the kinase on the glucose-induced transcriptional activation of the human insulin gene promoter in MIN6 cells. **Methods and Results:** In an *in vitro* phosphorylation study, PDX-1 was phosphorylated by PKC, but not by PKA or MAPK. Electrophoretic mobility shift assay (EMSA) and chloramphenicol acetyltransferase (CAT) assay using the human insulin gene promoter demonstrated that increased DNA-binding and transcriptional activities of PDX-1 induced by high glucose was blocked by calphostin C, an inhibitor of all PKC isoforms, but unaffected by PMA, an activator of classical and novel PKC, or Gö 6976, an inhibitor of classical and novel PKC. These results suggested that the PKC family which activated PDX-1 in MIN6 cells was atypical PKC. Western blot and immunocytochemical studies with anti-PKC ζ antibody confirmed the presence of PKC ζ in MIN6 cells. Furthermore, PKC ζ activity was significantly increased (2 fold, p<0.05) by stimulation with 20 mM compared with 2 mM glucose. **Conclusion:** High glucose induced phosphorylation and increased DNA-binding activity of PDX-1 by activating atypical PKC including PKC ζ, which in turn resulting in transcriptional activation of the human insulin gene promoter.

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GLP-1 PROMOTES DNA SYNTHESIS, ACTIVATES PI 3-KINASE AND INCREASES PDX-1 DNA BINDING ACTIVITY IN β(INS-1)-CELLS

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Aims: Glucagon-like peptide-1 (GLP-1) is a potent glucoincretin hormone and a potentially important drug in the treatment of type II diabetes. We have investigated whether GLP-1 may act as a growth factor in the β(INS-1)-cells and have studied the signaling pathways and transcription factors implicated in this process. **Methods:** Cell proliferation was assessed by tritiated thymidine incorporation measurements. We have examined the action of GLP-1 on the enzymatic activity of phosphatidylinositide 3-kinase (PI3-K). The DNA binding activity of transcription factors was investigated by electrophoretic mobility shift assay. mRNA measurements were performed using the Northern technique. **Results:** GLP-1 caused an increase in tritiated thymidine incorporation in β(INS-1)-cells and PI3-K activity in a dose-dependent manner non-additively with glucose. The PI3-K inhibitors wortmannin and LY294002 blocked the effects of GLP-1 on DNA synthesis. PDX-1 DNA binding activity was increased by GLP-1 at 3 or 11 mM glucose and the PI3-K inhibitor LY294002 suppressed the action of GLP-1 on PDX-1 DNA binding activity. GLP-1 and glucose alone did not change AP-1 DNA binding activity. However, they synergized to increase the activity of AP-1. GLP-1 also increased the expression level of PDX-1 and insulin mRNAs. Finally, GLP-1 increased the incorporation of tritiated thymidine in isolated rat islets. **Conclusion:** The results suggest that GLP-1 may act as a growth factor for the β cell via a PI3-K mediated event. GLP-1 could also regulate the expression of the insulin gene and genes encoding enzymes implicated in glucose transport and metabolism via the PI3-K/PDX-1 transduction signaling pathway.

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GLUCOSE POTENTIATES CYTOKINE-INDUCED MITOGEN ACTIVATED PROTEIN KINASE (MAPK) ACTIVITY IN RAT ISLETS.

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Glucose potentiates the effect of IL-1β on insulin release and nitric oxide (NO) production. Glucose is known to activate ERK1/2 and p38 in cell lines.We have previously shown that IL-1β caused activation of the MAPKs ERK1/2, p38 and JNK. The aim of this study was to investigate the effects of glucose on cytokine-induced MAPK activity. The hypothesis was that glucose potentiates cytokine-induced MAPK activity and that this signals the modulating effect of glucose on insulin release and NO production. For this purpose rat islets were precultured 4 h at 3.3 mM D-glucose and exposed to 0, 4, 20 or 60 U/ml IL-1β at 3.3, 5.5, 11 or 17 mM D-glucose (D-G). MAPK activity was measured by phosphotransferase assay using Elk1, ATF2 and Hsp25 as substrates. Accumulated insulin release was measured by ELISA and nitrite production by Griess reagent. Exposure to 4 and 20 U/ml IL-1β for 24 h had no effect while 60 U/ml IL-1β significantly increased insulin release at 3.3 and 5.5 mM D-G ($p<0.04$). At 11 mM D-G, exposure to 4 U/ml IL-1β had no effect while 20 and 60 U/ml significantly inhibited insulin release. At 17 mM D-G all IL-1β concentrations significantly inhibited insulin release. Increasing D-G concentration from 3.3 to 11 mM caused significant increases in IL-1β induced nitrite production at all IL-1β concentrations. Increasing the D-G concentration above 11 mM had no effect. Exposing islets to 3.3-17 mM D-G without IL-1β induced a significant increase in the phosphorylation of Elk1 but had no effect on ATF2 and Hsp25. Increasing D-glucose concentration from 3.3-17 mM caused a significant increase (Cuzicks test, $p=0.023$) in phosphorylation of Hsp25 induced by 4 U/ml IL-1β but did not affect phosphorylation induced by 20 or 60 U/ml IL-1β. We suggest that p38 activity may signal glucose potentiation of inhibition of insulin release and increase in nitrite production at low concentrations of IL-1β.

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ANTISENSE-RNA SUPPRESSION OF CA^{2+}/CALMODULIN DEPENDENT PROTEIN KINASE $II\delta_2$ (CaMK II) IN INS-1 CELLS CAUSES A LOSS OF INSULIN, GLUT-2, GLUCOKINASE AND IAPP GENE EXPRESSION

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Aims: CaMK $II\delta_2$ is highly expressed in INS-1 cells and colocalized with insulin secretory granules. The enzyme is activated in response to rising glucose levels in β-cells. To investigate its role in β-cells we suppressed CaMK $II\delta_2$ by using retroviral antisense mRNA expression. **Methods:** CaMK $II\delta_2$ was cloned in antisense direction into the retroviral expression vector p50-M-X-neo and 8 stable clones were established after infection of INS-1 cells. CaMK $II\delta_2$ and insulin Northern blots were performed by using digoxigenin labled probes. Other mRNAs were detected by semiquantitative PCR. For Western blot detection our rabbit CaMK IIγ/δ antibody was purified by stripping it with bacterially expressed CaMK IIγ. For insulin promoter assays the rat insulin-1 promoter (W. Knepel) was cloned into a luciferase vector. The IDX-1 antibody we obtained from J. Habener. **Results:** In antisense CaMK $II\delta_2$ cells sense CaMK $II\delta_2$ was no longer detectable while antisense CaMK $II\delta_2$ mRNA was overexpressed 20-50 fold. Western blots showed a 90% loss of the protein. Insulin was decreased from 90 µg/mg in wt INS-1 cells to 0-5 µg/mg in antisense cells. Insulin mRNA was reduced by more than 90% and insulin promoter activity by ~95%. Other mRNAs like Glut-2, glucokinase and IAPP the transcription of which is controlled similarly to insulin gene transcription also were greatly decreased - determined by semiquantitative PCR - while β-cell specific mRNA expression of GLP-1 receptor or calcineurin was not altered. The transcription factor IDX-1/PDX-1/IPF-1/STF-1 was present in the usual amount in all antisense cells as tested by Western blotting. In antisense cells MTS assays show a high metabolic activity at low glucose levels of 0.5 mmol/l which may be due to hexokinase taking over the function of glucokinase. The glucose independent insulin secretion seems not to be altered since the response to TPA was maintained. **Conclusion:** CaMK $II\delta_2$ seems to be involved in the transcriptional regulation of gene products neccessary for the glucose dependent insulin secretion.

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INSULIN SECRETION IN Ca^{++}/CALMODULIN KINASE $II\delta_2$ – DEFICIENT INS-1 CELLS

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Aims: To explore the role of Ca^{++}/calmodulin dependent protein kinase $II\delta_2$ (CaMK $II\delta_2$) in insulin secretion its synthesis was suppressed in INS-1 cells. CaMK $II\delta_2$ is the major CaMKII in INS-1 cells. is associated with insulin secretion vesicles and is phosphorylated upon glucose stimulation. **Materials and Methods:** CaMK $II\delta_2$ was stably suppressed by 90% at the protein level by a retroviral RNA antisense technique while CaMK IIγ was not altered (clone: INS-W12). LacZ controls contained ß-galactosidase in the same vector. Insulin was measured by RIA in supernatants of static incubations for 45 or 90 min. **Results:** Insulin content in INS-W12 cells was 5±2 µg/mg protein compared to 64±12 µg/mg in LacZ controls (n=4). Due to loss of glucokinase and of GLUT2, INS-W12-cells had a half max. glucose metabolism at 1 mM compared to 7 mM in controls. TPA (0.1 µM) increased insulin secretion by 63±8% in INS-W12 and by 240±56% in control cells. Glucose did not stimulate insulin release between 0-11 mM (4±5% at 11 mM, n=6) in INS-W12 but by 456±83% in control cells. Oxidative glucose metabolism was greatly stimulated by glucose in both cell types as shown by MTS-assays using antimycin. Insulin release after incubation for 2h in 0 mM glucose-KRB was dependent on mitochondrial metabolism and inhibited by 10 µM antimycin (58±13% of control, n=3) in INS-W12 but not in control cells. Diazoxide (DZX, 250 µM) decreased insulin release to 66±14% of DMSO control values in INS-W12 but had no effect on control cells at 0 mM glucose. K^+ 40 mM stimulated insulin release in the presence of DZX to 204±18% in INS-W12 and to 345±78% in INS-1 cells.**Conclusion:** Insulin secretion in response to activation of PKC and to Ca^{++} - elevation is maintained in the absence of CaMK $II\delta_2$ in INS-1 cells indicating that it does not directly regulate exocytosis although its quantity may be modulated by CaMK $II\delta_2$. Glucose responses. however, are lost although glucose is metabolized and insulin secretion requires mitochondrial metabolism in CaMK $II\delta_2$ antisense cells. CaMK $II\delta_2$ appears to interfere with nutrient secretagogue stimulated insulin secretion by interfering with mitochondrial metabolism and cellular energy stores.

OP 14
Hepatic Glucose Metabolism

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THE QUANTIFICATION OF GLUCONEOGENESIS IN HUMANS BY ^2H_2O AND [2-^{13}C]GLYCEROL YIELDS DIFFERENT RESULTS.

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Aim: There is no gold standard for the quantification of gluconeogenesis *in vivo*. During the past decade methods based on the use of stable isotopes are reported, from which the methods using ^2H_2O or [2-^{13}C]glycerol and the mass isotopomer dilution analysis of glucose are mostly used. Both methods do not involve assumptions regarding the enrichment of the oxaloacetate precursor pool. The aim of this study was to compare the results of both methods.
Materials and Methods: We measured gluconeogenesis in 6 healthy postabsorptive males after administration of ^2H_2O (5g/kg body water) and, on another occasion, during a primed (190 µmol/kg), continuous infusion (3.15 µmol.kg^{-1}.min^{-1}) of [2-^{13}C]glycerol. Endogenous glucose production was measured by a primed (24.6 µmol/kg) continous infusion (0.33 µmol.kg^{-1}.min^{-1}) of [6,6-$^2H_2$]glucose. **Results**: Endogenous glucose production was not different after ^2H_2O administration or during [2-^{13}C]glycerol infusion (12.2 ± 0.7 vs. 11.7 ± 0.3 µmol.kg^{-1}.min^{-1}, p=ns). However, gluconeogenesis measured after ^2H_2O administration and during [2-^{13}C]glycerol infusion was 7.4 ± 0.7 vs. 4.9 ± 0.6 µmol/kg/min, (p=0.03), representing ~60 vs ~41% of the endogenous glucose production, resp. There were no statistically significant differences between the glucoregulatory hormones (catecholamines, cortisol, C-peptide, insuline and glucagon) between both study days. **Conclusion**: Gluconeogenesis measured by ^2H_2O yields consistently higher results than measured by [2-^{13}C]glycerol. This discrepancy between both methods probably relates to conceptual problems in underlying assumptions in one or both methods.

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INCREASED GLUCONEOGENESIS IN TYPE 2 DIABETES: SUBSTRATE CONTRIBUTION AND MODULATION BY LIPOLYSIS.

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Aims: The contribution of gluconeogenesis (GNG) to endogenous glucose output (EGO) in type 2 diabetes, and its modulation by physiologic factors is still controversial. Materials and methods: We used the deuterated water (^2H_2O) technique to measure GNG in 29 type 2 diabetic patients (D, age=58±1 yrs, BMI=29.0±0.5 kg/m^2, fasting plasma glucose [FPG]=8.2±0.3 mM, [range 5.4 - 12.8]) and 9 nondiabetic subjects (C, age=38±3, BMI=30.6±1.6) after 16 h of fasting. The night before the study, subjects ingested enough ^2H_2O to bring body water enrichment to ~0.50% at the end of the study. Total fractional GNG was determined from the ratio of deuterium enrichment at C-5/C-2 in plasma glucose assayed in hexa-methylene-tetramine. Fractional GNG from substrates other than glycerol (*ie* lactate, pyruvate, alanine, *etc*) was also evaluated from the ratio C-6/C-2. At 9 am, a primed-constant, 3-h infusion of 6,6-^{2}H-glucose was administered to measure EGO. Results: In type 2 patients, EGO averaged 876±29 µmol/min, of which 68±2% (or 601±30 µmol/min) derived from GNG. Of the GNG flux, 150±22 µmol/min (or 25±2%) was from glycerol, the remainder from other substrates. In nondiabetic subjects, EGO was 760±42 µmol/min (p<0.05 *vs* D), of which 60±5% (or 427±48 µmol/min) was by GNG (p<0.01 *vs* D). In the whole dataset, fractional GNG was positively related to FPG (r=0.42, p=0.01). In 8 type 2 patients, acipimox (250 mg) was given to suppress lipolysis, and GNG/EGO measurements were repeated 2 h later. Following acipimox, plasma FFA fell from 0.65±0.10 to 0.12±0.02 mM, and glycerol decreased from 49±7 to 10±3 µM, while FPG and plasma insulin dropped slightly (from 8.1±0.4 to 7.1±0.3 mM and 8.8±0.9 to 7.3±1.4 µU/ml, respectively). EGO decreased by 129±40 µmol/min (p<0.02 *vs* baseline), which was mostly due to a fall in GNG (by 118±40 µmol/min, p=0.02); of this, 46±29 µmol/min was GNG from glycerol. Conclusions: We conclude that (1) GNG is increased in moderately hyperglycemic type 2 diabetic patients; (2) both glycerol and lactate/pyruvate/alanine contribute to the increase in GNG; and (3) short-term suppression of lipolysis has a small effect on GNG.

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EFFECTS OF GLYCEROL AND LIPID INFUSION ON HEPATIC GLYCOGENOLYSIS IN MAN.

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Aims: Both free fatty acids (FFA) and Glycerol are considered to stimulate gluconeogenesis and could be also involved in the regulation of hepatic glycogen stores. This study was designed to examine the effects of glycerol *per se* vs. combined increase of FFA and glycerol on liver glycogen breakdown in man.
Materials and Methods: After an overnight fast, healthy subjects (age: 25±2 yrs, BMI: 21.7±1.1 kg/m²) underwent 3 protocols: (I) glycerol infusion (GLYC; n=5; plasma glycerol: 1.48±0.19 mM at 9 h; p<0.005); (II) lipid/heparin infusion (LIP; n=7; glycerol: 0.48±0.03 mM, p<0.005; FFA: 2.5±0.1 mM; p<10^{-3}) and (III) 0.9% NaCl infusion (CON; n=7; glycerol: 0.19±0.02 mM; FFA: 0.5±0.1 mM). Hepatic glycogen concentrations were determined noninvasively by in vivo ^{13}C NMR spectroscopy (3T/80cm Medspec, Bruker, FRG). Net rates of glycogen breakdown (V_{out}) were calculated from linear regression of the decrease of liver glycogen within 9 h.
Results: Plasma glucose concentrations similarly decreased (p<0.005) from ~5.2 mM to ~4.8 mM during all 3 experimental protocols. Plasma insulin concentrations increased during LIP (0 min: 44±6 pM; 9 h: 52±6 pM; p=0.02), but declined during GLYC (0 min: 40±5 pM; 9 h: 22±3 pM) and CON (0 min: 46±6 pM; 9 h: 29±5 pM) (p<10^{-3} vs. 0 min; p<0.01 vs. LIP). Before the start of infusions, hepatic glycogen concentrations were similar in all groups (~222 mM) and declined within 9 h to 167±5 mM during GLYC (p<10^{-5}) and to 150±8 mM during CON (p<10^{-6}), but did not change during LIP (9 h: 206±6 mM, p<10^{-6} vs. CON, p<10^{-5} vs. GLYC). Plasma FFA elevation reduced V_{out} by ~85% (LIP: 23±12 vs. CON: 157±27 µmol/l liver·min; p<10^{-4}), whereas plasma glycerol elevation decreased V_{out} only by ~53% (72±11 µmol/l liver·min; p<0.01 vs. CON; p<0.05 vs. LIP).
Conclusions: These results suggest, that FFA are more effective than glycerol *per se* to reduce glycogen breakdown in man and might therefore play an important role in the regulation of hepatic glycogen stores.

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INFLUENCE OF OLEATE ON HEPATIC GLYCOGEN SYNTHESIS

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Aims. Free fatty acids are implicated as a causal factor of non-insulin dependent diabetes mellitus (NIDDM). In liver, they activate gluconeogenesis but little is known about their effect on glycogen synthesis.
Methods. We have studied: a) the effect of oleate on glycogen synthesis in isolated hepatocytes from fasted rats incubated under conditions to ensure high rates of glycogen production, i.e. in the presence of glucose, 2% serum albumin, and of amino acids to increase glycogen synthase *a* (GS*a*) activity by cell swelling; b) the glycogen content of livers and related parameters of rats that were fed for 3 weeks with a high-fat diet (25%; controls, 10% fat).
Results. Glycogen synthesis in hepatocytes increased from 71.1±4.9 to 85.3±5.4 in the presence of 1 mM oleate and decreased to 56.2±3.5 µmol/g dry mass of cells/h (n=11) in the presence of 2 mM oleate. Stimulation of glycogen synthesis was due to an increase (14%) in GS*a* with little effect on phosphorylase *a* (P*a*) activity. Inhibition of glycogen synthesis at 2 mM oleate as compared to 1 mM oleate was due to the combined effects of a 26% increase in P*a* and a 9% decrease in GS*a*. The decrease in glycogen synthesis could not be ascribed to a detergent-like effect of oleate as intracellular ATP was not altered by oleate. Production of ketone bodies also gave no indication for mitochondrial uncoupling.
In rats fed on the high-fat diet the glycogen content of the liver was about 60% of the value in the controls (n=7 in each group). The concentrations of intrahepatic glucose, glucose 6-phosphate, UDPglucose, amino acids and of portal insulin and glucagon were similar as in control animals. The ratio P*a*/P*a*+*b* was 0.65±0.04 in control rats and 0.74±0.05 in the high-fat group (p<0.05). GS*a*/GS*a*+*b* was unaffected by the diet (0.14±0.01 *vs.* 0.14±0.02).
Conclusion. Free fatty acids can inhibit hepatic glycogen synthesis by interfering with the activity of the enzymes involved in glycogen turnover. This may contribute to glucose intolerance in NIDDM.

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This abstract has been withdrawn

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Hepatic insulin sensitivity is the single most important regulator of glucose tolerance P. Båvenholm*, J. Pigon**, and S. Efendic**. Division of Medicine; Departments of Emergency and Cardiovascular Medicin* and Endocrinology**, Karolinska Hospital and Institute, Stockholm

Aims: We studied the contribution of hepatic and extra-hepatic insulin sensitivity and insulin responsiveness to the variation in glucose tolerance in Swedish middle-aged men with normal OGTT (n=29), impaired OGTT (n=10) and with mild diabetes (n=15).

Methods: Oral glucose tolerance and the insulin response (0-30 min) were assessed during OGTT. These three groups of subjects were well matched for age, BMI and lean body mass. Insulin sensitivity and glucose turnover were determined during a 450 min sequential two-step euglycemic insulin clamp (infusion of 0.25 and 1.0mU/kg/min). HPLC purified [6-^{3}H]glucose was used as a tracer and a step matched tracer infusion method was applied to prevent a decline in plasma glucose specific activity. The lean body mass was determined by the DEXA test.

Results: Hepatic glucose production (Ra, rate of appearence) was decreased during the low insulin infusion rate in subjects with normal and impaired OGTT and diabetes (1.20±0.60 vs 0.86±0.46 vs 0.57±0.31 mg\kg\min, $p<0.001$), and was suppressed at the high insulin infusion step. The respective rates of glucose infusion (M-values) necessary to keep normoglycemia (plasma glucose 5.1mM) during the high dose insulin infusion were 8.1±3.1 vs 5.3±3.2 vs 5.2±2.4 mg\kg\min ($p=0.004$). Using stepwise regression analysis, 41 % of the variability in glycemic responses during OGTT was explained by the magnitude of the decrease in Ra during the low insulin infusion rate, whereas only 18% was accounted for by extra-hepatic insulin sensitivity (M-value) and 12% by the insulin response. Furthermore, in the diabetic subgroup delta Ra (increase in R^2 0.30) and body mass index (increase in R^2 0.22), together with insulin response (increase in R^2 0.14) predicted as much as 69% of the variation in OGTT. In healthy subjects only delta Ra made an independent contribution to the variability in OGTT (Multiple R^2 0.22).

Conclusion: In conclusion, we demonstrate that hepatic insulin sensitivity plays the most important role in determination of oral glucose tolerance.

OP 15
Diabetic Foot

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THE INCIDENCE AND RELATIVE RISK FOR FOOT ULCERATION IN TYPE II DIABETES.

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Aims: The incidence of foot ulcers in newly diagnosed (n = 31) and long-standing (n=156) type II diabetic patients was determined in a 4-year prospective study. To calculate the relative risk for foot ulceration, subjects were assigned to an exposed and non exposed group by their peripheral sensitivity and plantar load. **Materials and Methods:** In all, 187 subjects were recruited out of a large diabetes outpatient's department. Exclusion criteria were: history of foot ulceration, claudication, amputation, and severe foot deformities. Because clinically relevant parameters such as gender, HbA1c, neuropathy-status, foot deformities and plantar pressure did not differ between newly-diagnosed and long-standing subjects at baseline, their data were pooled for the outcome analysis. The exposed group was defined by a pathologically increased vibration perception threshold > 30 V plus a pathologically elevated forefoot plantar pressure. **Results:** The total observation time was 470.4 person-years. Seven exposed and 3 non-exposed patients (1 (3.2 %) newly-diagnosed and 9 (5.7 %) long-standing subjects) developed 18 neuropathic foot ulcers. The cumulative incidence of first foot ulceration for the whole study population was 5.9 % (95 % CI 2.5 – 9.3). The incidence density of all foot ulcers (including reulceration in the same subject) was 38.3 / 1000 person-years (95 % CI 34.5 – 42.2) and of all foot lesions (including 3 subjects who developed infected interdigital fissures or apical blisters) was 44.6 / 1000 (36.9 – 45.9). The relative risk for the exposed group to develop an ulceration was 14.7. **Conclusions:** A risk classification system based on the vibration perception threshold and on plantar pressure is a powerful instrument to define patients at-risk for foot ulceration. Since newly diagnosed patients were not different from long-standing subjects in terms of baseline risk-factors and ulcer development, they should be screened and treated in the same way as long-standing diabetic subjects.

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PROSPECTIVE RISK FACTORS FOR DIABETIC FOOT ULCERATION IN A LARGE POPULATION SAMPLE.

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Aims: A specialist diabetic foot screening programme was established in the primary care setting in the UK in order to identify risk factors in a large, prospective study. **Methods**: 9,710 diabetic patients were assessed for demographic, medical and social history, neuropathy symptom score, neuropathy disability score (NDS), monofilaments (MF), foot deformity score (FDS) and pedal pulse palpation. Patient risk of foot ulceration was categorised as 'high', 'medium' or 'low'. Two years after baseline screening, the incidence of new foot ulcers was assessed via postal questionnaires then confirmed via podiatry records. **Results**: 6,698 (69%) questionnaires were returned (403 patients had died, 201 had moved away and 2408 did not reply) and results are presented for patients with verified ulcer status (n=6,548). Mean age at baseline was 61.7 +13.3 years and diabetes duration, 5.0 (4.9, 5.2) years. 1,378 patients (21%) had peripheral neuropathy (NDS≥6/10) and 1,294 (20%) peripheral vascular disease (≤2/4 pulses). During the 2 year follow-up 284 patients developed new foot ulcers (2.2% annual incidence). Baseline variables predictive of foot ulcers were: lower limb amputation (Relative Risk: 9.5 [6.3, 14.2; 95% CI]), NDS≥6/10 (6.4 [5.0, 8.1]), abnormal pin-prick (5.0 [3.9, 6.4]), abnormal vibration sensation (4.9 [3.8, 6.4]), absent ankle reflexes (5.0 [3.7, 6.8]), insensitivity to 10g-MF (4.5 [3.6, 5.7]), reduced foot pulses (2.9 [2.3, 3.7]) ($p<0.001$). Socio-economic class, smoking history and alcohol consumption were not predictive of foot ulceration. Forward stepwise multiple regression identified diabetes duration, amputation, NDS≥6/10, 'high risk' category, insensitivity to 10g-MF, regular chiropody treatment, reduced palpable pulses, age, abnormal ankle reflexes and increasing FDS, in ranking order, most strongly predicted foot ulceration ($p<0.05$). **Conclusions**: This very large, prospective study confirms that simple clinical measures of peripheral neuropathy, in particular the NDS, are the best predictors of diabetic foot ulceration.

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FACTORS ASSOCIATED WITH BONY REGROWTH FOLLOWING PARTIAL FOOT AMPUTATION

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Aims: Hypertrophic bone formation has the potential to cause abnormal foci of high plantar pressure which may increase risk for reulceration and reamputation. However, we are unaware of previous studies evaluating risk factors for this entity. Therefore, the purpose of this study was to evaluate risk factors associated with bony regrowth following partial foot amputation. **Materials and Methods:** The records of 92 consecutive diabetic subjects with a mean age of 52.7 ± 6.0 years and a history of isolated partial ray amputation were abstracted from the University of Texas Diabetic Foot Registry. All subjects received repeat radiographs a mean 22.1 ± 6.1 months after the preliminary procedure. Hypertrophic bone formation was defined as > 3mm of bony regrowth. **Results**: A total of 44.6% of subjects reviewed had hypertrophic bone formation a mean two years following their isolated partial ray amputation. Factors significantly associated with this regrowth included male gender (58.1% vs. 16.7%, $p<0.01$, $X^2=14.0$, Odds Ratio = 6.9, 95% CI = 2.3 – 20.4) the use of manual bone cutting instrumentation (74.2% vs. 29.5%, $p<0.01$, $X^2=16.6$, Odds Ratio = 6.8, 95% CI = 2.6 – 18.1), and osteotomy made distal to the surgical neck of the metatarsal (34.1% vs. 11.8%, $p<0.02$, $X^2= 6.7$, Odds Ratio = 3.9, 95% CI = 1.3-11.3). There was not a significant association between regrowth and age, degree of glucose control, renal disease, body mass index, nutritional status, peripheral vascular disease, or previous amputation history. **Conclusions**: The results of this study suggest that the use of manual instrumentation, male gender, and metaphyseal amputation level may be associated with long-term bony regrowth following isolated ray amputation. The use of power instrumentation when performing these procedures may lead to a lower incidence of this potential reaction, thereby potentially reducing the risk for ulceration, infection, and reamputation.

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ULTRASOUND BONE DENSITOMETRY OF THE HEEL AND ITS RELATIONSHIP WITH THE BIOCHEMICAL MARKER OF BONE TURNOVER IN PATIENTS WITH CHARCOT OSTEOARTHROPATHY

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The risk of pathological fractures in the early stage of Charcot osteoarthropathy (CO) is high and therefore may be helpful to compare methods of bone turnover and heel bone densitometry. **Aims:** The aim of the study was to assess the heel ultrasound parameters in Charcot´s and non-Charcot´s foot and to compare it with the parameter of bone resorption - collagen type I cross-linked C-telopeptides (ICTP). **Patients and Methods:** Both bone-specific diagnostic methods were assessed in 16 diabetic patients in the early stage of CO with a mean age of 51 ± 13 years and disease duration 16 ± 7 years which did not differ from 30 sex- and age-matched healthy control subjects. Quantitative ultrasound densitometry (QUS) of a heel was made on a Lunar Achilles QUS device and T-score (a multiple of standard deviation of young adults of the same gender) of densitometric parameter stiffnes was included into analysis. For the description of bone resorption, ICTP assessed by Radioimmunoassay Kit, Orion Diagnostica, Finland (normal range 1.8 - 5 ug/l, variation coefficient 6%) was used. **Results:** A significant difference between patients with CO and the control group was found in ICTP (8.49 ± 4.37 vs. 3.94 ± 2.38 ng/ml, $p < 0.001$) which confirms active bone resorption in patients with CO. Ultrasound bone density (T-score of stiffness) was significantly lower in Charcot´s foot comparing with non-Charcot´s foot (- 3.00 ± 1.9 vs. - 2.40 ± 1.32, $p < 0.05$), no significant difference between right and left foot was found (- 2.59 ± 1.4 vs. - 2.82 ± 1.9). A significant correlation was demonstrated between ICTP and stiffness ($r = - 0.73$, $p < 0.01$) in Charcot´s foot. **Conclusions:** A significantly lower ultrasound bone density in Charcot´s foot correlating with increased bone resorption confirm an increased risk of fracture in patients in the early stage of CO .

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A FORCE-PRESSURE DEVICE IS ABLE TO DETECT ABNORMAL SHEAR FORCE PATTERNS IN THE DIABETIC NEUROPATHIC FOOT

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Aims: Diabetic neuropathy has been widely investigated from a biomechanical point of view. Pressure measurement devices have detected abnormal pressure distribution, and force platforms abnormal overall force patterns. The innovative instrument used in this study allows the simultaneous detection of pressure and all the components of the overall ground reaction during gait. Therefore shear force patterns have been recorded together with the specific values in correspondence with different subareas like the heel and the metatarsal heads. **Materials and Methods:** A prototype of a piezo-dynamometric platform has been used. It detects, for each sample, the force vector, its point of application, the free moment, and the pressure distribution under the foot. Local components of vertical and shear forces and free moment are reliably estimated for each foot subarea of interest, of any shape and size. Four groups of patients were evaluated: Controls (C=15), Diabetics without (D=14) and with neuropathy (DN=15) and Diabetics with previous neuropathic foot ulcer (DPU=10). **Results:** Mean integrals and mean peaks of the vertical forces were similar in C and D, and significantly increased in DN and DPU ($p<0.01$). Even more remarkable differences were found for the medio-lateral forces: force peaks increased for metatarsal and decreased fo hallux in DN and DPU in respect of C ($p<0.01$).

Table 1. Mean ranges (max-min values) of shear forces under the metatarsals and hallux. Forces are expressed as % of body weight.

	METATARSALS				HALLUX			
	C	D	DN	DPU	C	D	DN	DPU
Ant-post	16.2±1	15±1	14±1	17.8±1	5.6±.8	4.4±.7	3.5±.5	3.6±.9
Med-lat	4±.4	4.1±.3	4.9±.4*	5.1±.5*	1.6±.4	1.1±.3	.9±.3*	.9±.3*

Conclusion: The results of this investigation seem to support that the shear stress is altered in patients with peripheral neuropathy and may have a role in the pathogenesis of foot ulceration in these patients. Interestingly the medio-lateral component of the shear stress was more altered than the antero-posterior component especially under the metatarsals. The measurement device we used has proved essential for the complete characterization of specific foot subareas in terms of local forces and moments.

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TREATMENT WITH A HUMAN SKIN EQUIVALENT, GRAFTSKIN (APLIGRAF®), IMPROVES HEALING RATE IN DIABETIC FOOT ULCERS

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Aims. To assess the ability of Graftskin (Apligraf®), a Human Skin Equivalent to improve the median time to wound closure and the wound closure rate in non-ischemic, non-infected diabetic foot ulcers. **Methods.** This was a prospective, randomized, placebo-controlled study. Patients in the Graftskin group received treatment material once a week for a maximum of four weeks (five applications). Graftskin treatment was compared to a control treatment consisting of woven gauze kept moist by saline. Proper wound care, including extensive debridement and weight off loading was provided to all participants. **Results.** Sixteen patients were randomized to Graftskin application and 17 to control treatment. The two groups were matched for age, sex, diabetes duration, and ulcer size and duration. The Kaplan-Meier median time to complete closure was 38.5 days for Graftskin, significantly lower than the 91 days observed in the control group ($p<0.01$). Complete wound closure was achieved in 12 (75%) Graftskin treated patients compared to 7 (41%) control-treated patients ($p<0.05$). There were no significant side effects related to Graftskin treatment. **Conclusions.** Weekly application of Graftskin, a living human skin equivalent, in diabetic foot ulcers for a maximum if four weeks results in a higher healing rate when compared to state of the art currently available treatment. Treatment with Graftskin was not associated with any significant side effects and may prove to be a very useful adjunct for the management of chronic diabetic foot ulcers, which are resistant to the currently available standard care.

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The Treatment of Indolent Neuropathic Ulceration of the Diabetic Foot with Hyaff[R] an ester of Hyaluronic Acid

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Neuropathic ulceration is a common complication of the diabetic foot and ulcers associated with sinus formation and bone exposure are hard to heal. A new biomaterial Hyalofill[R] (Convatec Ltd) for treatment of indolent ulcers has recently been introduced. Hyalofill consists 100% of Hyaff an ester of Hyaluronic Acid. When applied to the wound it degrades thus providing a hyaluronic acid rich tissue interface. This may promote granulation tissue and speed healing. The aim of our study was to investigate whether Hyalofill could promote the healing of neuropathic ulcers with exposed bone and sinus formation. We studied 30 patients with indolent neuropathic ulcers. Fifteen patients were treated with Hyalofill plus standard treatment (active group) and 15 received standard treatment alone (controls). In the active group, mean age was 58 ± 12 years (mean ± SD), mean duration of ulceration 45 ± 55 weeks. There were 13 ulcers with sinuses and 13 with bone exposed. Sites of ulceration were: toe 1, metatarsal 11, heel 2 and rocker-bottom (Charcot) 2. In the control group, mean age was 55 ± 12 years, mean duration of ulceration 48 ± 64 weeks. There were 9 ulcers with sinuses and 9 with bone exposed. Sites of ulceration were toes 4, metatarsal 7, heel 2, rocker-bottom 2. In the active group 12/13 sinuses healed compared with 1/9 in the control group ($p>0.01$). In the active group, 10 of the 15 ulcers healed, compared with 3 out of 15 in the control group ($p<0.05$). We conclude that Hyalofill application results in a higher degree of closure of sinuses and improved healing of indolent neuropathic ulcers

OP 16
Experimental Immunology II

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THYMIC INSULIN-RELATED POLYPEPTIDES IN DIABETES-PRONE BIO-BREEDING RATS

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Aims. Insulin-like growth factor 2 (IGF-2) is the dominant member of the insulin family expressed in the thymus of different species. In the human thymus, the expression of the IGF-2 gene is under the control of P3 and P4 promoters active in fetal and extrahepatic adult tissues (J. Neuroendocrinol., in press). Antibodies (Ab) directed to IGFs or IGF receptors influence early T-cell development in murine fetal thymic organ cultures, whereas Ab to proinsulin have no effect (Eur. J. Clin. Invest., in press). This study investigates the hypothesis that a defect of thymic IGF presentation could be involved in the pathophysiology of Bio-Breeding (BB) rat autoimmune diabetes.

Materials & Methods. The expression of IGF-1, IGF-2, proinsulin, and actin genes was investigated by RT-PCR (30 cycles) in the thymus of diabetes-prone and diabetes-resistant BB rats. RT-PCR were performed on rat tissues from different ages: 2 days, 5 days and 5 weeks. For each series, liver and brain were also collected and used as control tissues.

Results. IGF-2 transcripts were not detected by RT-PCR in the thymus of 11/15 diabetes-prone BB rats, but were clearly identified in the thymus of 15/15 diabetes-resistant BB rats. The defect of thymic IGF-2 expression was evidenced at different ages. The expression of proinsulin and IGF-1 genes was normal in the thymus of diabetes-prone and diabetes-resistant BB rats. The defect of IGF-2 expression was thymus-specific since IGF-2 transcripts were detected in the brain and liver of diabetes-prone BB rats. These results confirm previous observations at the protein level.

Conclusions. IGF-2 is the dominant thymic insulin-related polypeptide involved both in the control of T-cell differentiation and central self-tolerance. The defect of IGF-2 expression in the thymus of diabetes-prone BB rats could contribute to the lymphopenia of these animals, as well as to the absence of central T-cell tolerance of insulin-secreting islet ß cells.

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DISORGANIZATION OF THYMIC MEDULLA PRECEDES EVOLUTION TOWARDS DIABETES IN FEMALE NOD MICE

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Aims: To study the structure of thymic medulla in autoimmune prone mice. **Materials and Methods**: mAb 95 which specifically recognises one subset of medullary epithelial cells in control mice was used to analyse cryosections of thymuses from NZB (n=3) and NOD mice (n=27). This mAb allows to quantify the scattering of medullary epithelial cells in the cortex (AMC value) and the size of thymic medulla (MA/TA). **Results:** In control mice MA/TA and AMC were 0.24 ± 0.03 and 0.94 ± 0.44 respectively, in contrast in medulla-less thymuses from RelB-deficient mice which develops a polyinflammatory disease they were 0.01 (no more medulla) and 15.89 ± 5.88 respectively (high disorganization). Thymuses from NZB mice in which medullary foci can be found (MA/TA=0.07 ± 0.02), cortex contain scattered medullary epithelial cells (AMC=12.52 ± 0.42). In NOD male mice, medulla was reduced (MA/TA=0.09 ± 0.06) and some scattered medullary epithelial cells can be found in the cortex (AMC=4.93 ± 1.79). The severity of disorganization of thymic medulla was shown to correlate with the subsequent evolution towards diabetes in female hemithymectomized NOD mice. Seventy day-old diabetes-prone female mice showed a combination of reduced medulla (MA/TA=0.15 ± 0.04) and high AMC value (9.99 ± 4.3), non diabetes-prone mice showed a lower AMC value (6.55 ± 4.02) with a less reduced medulla (MA/TA=0.2 ± 0.06). Non-major histocompatibility complex (MHC) NOD genes mainly control this phenotype since C57/BL6 H-2^{g7} congenic mice have a normal medulla. It persists in conditions where effector lymphocytes that lead to diabetes are inhibited in periphery. **Conclusion**: A thymic phenotype was identified in autoimmune prone mice which combines a smaller and partially disorganized thymic medulla and is linked to the intensity of the disease. These results suggest that alteration of thymic stroma might play a role in the progression towards autoimmune disease in NOD mice.

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ANTIGEN EXPRESSION ON ß-CELLS OF LONG-TERM CULTURED AND FRESHLY-ISOLATED ISLETS: EFFECT OF INF-γ

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Aim: Although long-term culture decreases immunogenicity of islets due to a loss of „passenger" leukocytes permanent acceptance is not necessarily observed after allogeneic transplantation. Since cell-mediated recognition of foreign cells is influenced by surface antigens, we investigated whether antigen expression of ß-cells is changed by long-term culture and whether INF-γ is able to increase antigen expression of cultured islets. **Materials and Methods:** Islets isolated from 10d old LEW.1A rats were cultured at 37°C for 14d (t_{14}) or 28d (t_{28}). Precultured islets or freshly-isolated islets (controls, t_0) were then incubated for 2d without or with rat rINF-γ (1000IU/ml). After preparation of single cells antigen expression was determined by double labelling with monoclonal antibodies (OX6: MHC II; OX18: MHC I; 1A29: ICAM-1; K14D10: ß-cells) and measured on a flow cytometer. **Results:** Using freshly-isolated islets (n=10) INF-γ leads to induction of MHC II on ß-cells (1.0±0.4% vs. 31.8±3.0%, $p<0.001$), to an increased MHC I antigen density (3.8±0.9logU vs. 43.9±7.8logU; $p<0.001$) and to an increase of ICAM-1$^+$ß-cells to 15.5±1.9% ($p<0.05$). The preculture (n=10) resulted in a decrease of ICAM-1$^+$ß-cells (t_0: 10.2±1.0%; t_{14}: 5.6± 0.6%; t_{28}: 4.3±0.8%), and had no effect on MHC I and II expression. INF-γ induced MHC II on ß-cells also after preculture of islets, but in a reduced extent (t_{14}: 11.8±1.0%; t_{28}: 10.5±1.3%). MHC I antigen density was enhanced by INF-γ as observed with freshly-isolated islets and was not significantly different. INF-γ was not able to enhance ICAM-1$^+$ß-cells after preculture (t_{28}: 4.3±0.8% vs. 5.5±1.1%). **Conclusions:** Antigen expression on ß-cells is partly reduced by long-term culture of islets, but stimulation by INF-γ, which is one cytokine released by graft invading lymphocytes, was not prevented. Therefore, we conclude that immunogenicity of islets seems to depend not only on the presence or absence of „passenger" leukocytes but also on antigen expression of the grafted islets, which is important for recognition by alloreactive lymphocytes.

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DIFFERENTIAL EFFECTS OF GLUCOSE ON THE PRODUCTION OF TUMOR NECROSIS FACTOR AND INTERLEUKIN-1β

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An important modulatory effect of glucose on the immune system has been suggested by recent in vitro studies, including stimulation of synthesis of the proinflammatory cytokines tumor necrosis factor-α (TNF) and interleukin-6 (IL-6). **Aims:** to investigate the effect of glucose on spontaneous and lipopolysaccharide (LPS)-stimulated production of the proinflammatory cytokines TNF and IL-1β, in vitro and in vivo. **Materials and Methods:** peripheral blood mononuclear cells isolated from 5 volunteers were stimulated in vitro with LPS (1 ng/ml) (24h at 37°C), in the presence of increasing glucose concentrations (5 to 30 mmol/L). In addition, hypoglycemia was artificially induced in 4 volunteers by continuous infusion of insulin intravenously. At baseline (before start of insulin), and at glucose concentrations of 5.0, 3.5 and 2.5 mmol/L, blood samples were collected and circulating concentrations, as well as spontaneous and LPS-stimulated production of cytokines in a whole-blood assay were assessed. **Results:** After stimulation of cells with LPS in vitro, there was a significant dose-dependent increase in the synthesis of TNF (up to a maximum 78% increase, $p<0.03$). Neither the LPS-induced production of IL-1β, nor the spontaneous release of cytokines, were influenced by hyperglycemia. After induction of artificial hypoglycemia in vivo, no effect of glucose concentrations on circulating levels or spontaneous cytokine release was observed. However, the decrease in glucose concentrations resulted in a significant reduction of LPS-induced TNF synthesis (86% of baseline production at 5.0 mmol/L glucose, 40% production at 3.5 mmol/L, and 22% production at 2.5 mmol/L, $p<0.01$). In contrast, no significant effect of hypoglycemia was exerted on LPS-stimulated IL-1β (86% of baseline production at 5.0 mmol/L glucose, 112% at 3.5 mmol/L, and 68% at 2.5 mmol/L, $p>0.05$). **Conclusions:** glucose concentrations modulate the stimulated TNF production capacity, but have no effect on IL-1β synthesis. Because TNF has an important role in both the pathogenesis of type I diabetes, as well as in the resistance to insulin, the potential clinical importance of this phenomenon deserves further investigation.

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SULFATIDE STABILIZES INSULIN CRYSTALS AND, AT NEUTRAL pH, MEDIATES CONVERSION OF INSULIN HEXAMERS TO MONOMERS

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Aim: To investigate whether an interaction exists between insulin and sulfatide (3'-sulfogalactosylceramide). Sulfatide is present in the secretory granules and at the surface of beta cells, and anti-sulfatide antibodies are found in high titres in patients with insulin-dependent diabetes. **Methods:** An ELISA assay was used to show and map the binding of sulfatide to insulin. Light and electron microscopy were used to quantify distortion of insulin crystals. Thin layer chromatography (TLC) was used to analyse the association state of insulin. **Results:** We demonstrate here that sulfatide binds to human insulin. This binding was found to involve A8-A10 (insulin A-chain amino acid in position 8-10) since labelling with a monoclonal antibody to this domain was reduced with 42% ($p=0.004$) in the presence of sulfatide. In contrast, labelling with monoclonal antibodies to A15, B3 and B25-B30 were not affected. Also functionally, sulfatide interacts with insulin. At pH 5.5, similar to that of beta-cell secretory granules, sulfatide substantially reduced degradation of pre-made insulin crystals; after 24 hours, complete distortion of pure insulin crystals were seen, whereas, in presence of sulfatide 90% of the crystals were conserved. 50% of these were still preserved after 8 days, and complete distortion was not seen before 21 days. At neutral pH, similar to that of beta-cell surface, TLC of dissolved insulin showed, that sulfatide mediated conversion of hexa- and dimer insulin towards the biological active monomer insulin conformation. That sulfatide mediates formation of insulin monomer at neutral pH was further supported by massive insulin fibrillation upon adjusting pH to 5.5, a phenomenon, which is never seen with hexamers. The precursor of sulfatide, galactosylceramide, showed no binding or interaction with insulin. **Conclusion:** Thus, for the first time, a genuine beta-cell component, sulfatide, is found to act as a molecular chaperone by stabilizing the insulin crystals under conditions similar to that of the secretory granules and, at neutral pH, by mediating dissociation of insulin hexamers towards monomers.

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ANTI-SULFATIDE ANTIBODIES IN IDDM DECREASE INSULIN EXOCYTOSIS

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Anti-sulfatide antibodies (ASA;IgG) are found in the majority of newly diagnosed IDDM (type 1) patients as well as in prediabetic persons. The aim of the study was to investigate the influence of ASA and sulfatide (3'-sulfogalactosylceramide) on beta-cell exocytosis. Incubation of rat islets with the monoclonal anti-sulfatide antibody Sulph I (20 µg/ml) for 24 h resulted in 37% reduction of insulin secretion. Patch-clamp techniques and capacitance measurements of exocytosis were used to investigate how Sulph I affects insulin secretion. Pre-incubation of rat beta cells with Sulph I for 0.5 h reduced Ca^{2+}-evoked exocytosis by 37% which was not associated with a change in the activity of the voltage-gated Ca^{2+} channels or the ATP-sensitive K^+-channels. No change in the exocytotic capacity was observed for boiled Sulph I or for a monoclonal antibody against a sulfatide precursor. Interestingly, ASA-positive serum from newly diagnosed IDDM patients reduced Ca^{2+}-induced exocytosis by 42%. Exocytosis was not affected by ASA-negative IDDM or control serum. Furthermore, ASA-positive IDDM serum washed through a protein A column did not affect exocytosis, whereas the ASA effluent decreased the exocytotic capacity of the beta cells with 35%. These data suggest that sulfatide is involved in the insulin secretion process. This is consistent with the presence of sulfatide in the secretory granules and at the surface of the beta cells. In addition, sulfatide stimulated Ca^{2+}-dependent exocytosis by 180%. We speculate that ASA in pre- and newly diagnosed IDDM patients reduce the sulfatide concentration and lead to an impaired secretory response. Thus, for the first time a specific antibody, associated with IDDM, and the corresponding genuine antigen have been demonstrated to affect beta-cell exocytosis.

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Influence of immunization with an HLA-DR3 restricted GAD65 peptide on low-dose STZ diabetes in an HLA-DR3/huCD4 transgenic mouse model.

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Aims: Immune responses to glutamic acid decarboxylase 65 (GAD65) are known to precede manifestation and accompany progression of autoimmune diabetes. Using an DR3[DR17]$^+$ / hu CD4$^+$ / mu cd4$^-$ multiple transgenic mouse model, we tested whether immunization with the peptide covering the HLA-DR3 restricted sequence $GAD65_{256\text{-}267}$ (GAD-Pep) lead to hyperglycemia by itself, or may accelerate onset of streptocotocin (STZ) induced diabetes.
Material and methodes: Mice were i. p. immunized with GAD-Pep emulsified in CFA and/or IFA. Ten days later these mice, and respective control animals, were treated with 40mg/kg STZ for five consecutive days (low-dose STZ), and blood glucose levels were monitored.
Results: STZ induced hyperglycemia was evident around day twelve after the first injection of the diabetogenic drug STZ. In three independent experiments, preceding injection with GAD-Pep emulsified in IFA or CFA, accelerated onset of diabetes in single mice (2 of 3, 3 of 4, and 1 of 4, respectively). Hyperglycemia occurred 3 to 5 days earlier than in STZ-treated control animals. Interestingly, animals immunized with GAD-Pep emulsified in CFA and boostered with peptide in IFA, were seemingly protected as compared to STZ treated controls. Immunization with GAD-Pep, using IFA or CFA as adjuvant, was not sufficient to induce hyperglycemia by itself.
Conclusion: Using transgenic mice, we show here that immunization with a HLA-DR3 binding peptide derived from the potential autoantigen GAD65 may increase the susceptibility of DR3 bearing transgenics to develop autoimmune diabetes. Apparently, altered susceptibility is greatly influenced by the immunization protocol. Importantly, induction of immune reactivity to putative pathogenetic peptide sequences alone is not sufficient to induce diabetes, rather, further diabetogenic stimuli are required.

OP 17
Islet and Pancreas Transplantation

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REDUCED SUSCEPTIBILITY OF LARGE MAMMAL ISLETS TO HUMAN CYTOKINE TOXICITY.

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Aims: The potential benefits of islet xenograft in Type 1 diabetes (IDDM) include the possibility that xenoislets may be less susceptible to autoimmune attack. Cytokines play a major role in the pathogenesis of IDDM by causing alteration of insulin release (IR) and cytotoxicity. We evaluated the function and survival of isolated human (HI) and bovine (BI) islets exposed to a combination of 50 U/ml rh-interleukin 1-beta, plus 1,000 U/ml rh-tumor necrosis factor alpha, plus 1,000 U/ml rh-interferon gamma. Materials and Methods: Islets were prepared from 4 human and 8 bovine pancreases by collagenase digestion and density gradient purification, and IR was evaluated after 24h exposure to the cytokines (CKs); islet cell survival was assessed by the annexin V and propidium iodide method after 48h exposure of dispersed HI and BI cells to the CKs mixture; RT-PCR studies were performed to evaluate islet expression of mRNA encoding for Bcl-2, an antiapoptotic protein. Results: upon CKs exposure, IR from HI increased at low glucose, and decreased at increased glucose concentration (table 1). With BI, the increase of IR at low glucose was accompanied by maintained IR at high glucose, with overall preserved capacity to sense the glucose changes (table 1). Incubation with the cytokines induced apoptosis in HI (apoptotic cells: 39±5% in exposed cells vs 13±2% in control cells, $p<0.01$) but not in BI (apoptotic cells: 17±2% in exposed cells vs 12±2% in control cells, NS), and therefore more BI than HI cells exposed to Cks survived (79±6% vs 54±4%, $p<0.01$). Accordingly, Bcl-2 down regulation was detected in HI, but not in BI, pre-exposed to the CKs mixture. Conclusions: BI are less susceptible than HI to human cytokine induced damage, which may turn to be a potential advantage of xenotransplantation.

	IR (pg/islet/45min)	
	3.3 mM glucose	16.7 mM glucose
HI (control)	23.9±3.8 (n: 6)	104±9.1 (n: 7)*
HI+cytokines	46.7±4.8 (n: 9)#	48.7±5.8 (n: 9)#
BI (control)	26.2±2.9 (n: 17)	130.2±15.7 (n: 18)*
BI+cytokines	73.4±7.6 (n: 15)#	119.6±10.5 (n: 16)*

** $p<0.01$ vs 3.3 mM glucose and # $p<0.01$ vs control*

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PROLONGED SURVIVAL OF RAT-ISLET XENOGRAFTS IN MICE BY CD45RB ANTIBODY MONOTHERAPY

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Anti-thymocyte globulin which contains antibody's against CD45 has proved to be one of the most efficacious agents in preventing rejection. Recently, anti-CD45RB alone has been shown to prolong kidney and islet allografts survival in mice. **Aim** of the present study is to investigate whether anti-CD45RB therapy is effective in preventing rejection of islet xenografts. **Materials and Methods**: Streptozotocin diabetic C57B6 mice received one thousand AO-rat islets under the kidney capsule. The mice in the experimental group received 100 µg CD45RB i.v. on day -1, 0 and 5. Control xenograft recipients remained untreated. **Results:** All animals became normoglycemic within two days postimplant. Untreated control animals returned to hyperglycemia within 7 days postimplant (n=4). The CD45RB animals (n=6), however, survived for prolonged periods of time ($P < 0.01$) and returned to hyperglycemia on day 17, 17, 26, 27, 45, and 54. To get more insight in the mechanisms responsible for the prolonged graft survival, we compared grafts of treated and nontreated animals at day 6 after implantation. Nontreated control grafts were heavily infiltrated by lymphocytes while the grafts of CD45RB treated animals only contained some lymphocytes in the periphery of the islets but virtually no infiltration. The grafts of nontreated controls contained more T-cells and B-cells than the grafts of treated animals. Also, in the grafts of controls the lymphocytes were more activated as evidenced by a higher CD25 expression. We found no expression of IL-2 or IL-4 mRNA (as measured by RT-PCR) in the grafts while the expression of IFN-γ and IL-10 was virtually the same in treated and nontreated grafts. Our data show that CD45RB can effectively prolong the survival of islet-xenografts. Our present research is focussing on the optimalization of CD45RB-treatment on islet-xenograft survival.

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DONOR AGE DOES NOT INFLUENCE THE OUTCOME OF ISLET TRANSPLANTATION.

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Aim: Old donor age has been considered as a risk factor and relative contraindication for transplantation. In this study, we investigated the characteristics and function of islets isolated from different donor age.
Methods: Islets were isolated from 8-week (I-A), 32-week (I-B) and 64-week (I-C) C57BL/6 mice by collagenase digestion method. Three hundred islets were syngeneically transplanted under left kidney capsule of streptozotocin-diabetic recipients (R-A, R-B and R-C, respectively).
Results: The islet number of I-A, I-B and I-C was 97±4, 219±22 and 281±19 islet/mouse, respectively. ($P<0.05$) The islet area in I-A, I-B and I-C was 0.019±0.002, 0.024±0.002 and 0.026±0.001 mm^3, respectively. ($P<0.05$) I-B and I-C contained more insulin (0.81±0.06 and 0.92±0.07 μg/150 islets, respectively) than I-A (0.47±0.09 μg/150 islets). ($P<0.05$) However, I-A and I-B had similar stimulation index in the first and second phase of insulin secretion. After transplantation, the fall of blood glucose in R-C was faster than that of R-A and R-B but the increase of body weight was similar in 3 groups. At 12 weeks after transplantation, the blood glucose, body weight and HbA1c were similar in all groups. However, the R-C had better glucose tolerance than R-A. ($P<0.05$) Normoglycemia could be maintained lifelong in R-A (43±4 weeks) and R-B (42±4 weeks).
Conclusions: The islets isolated from donors with different age have different characteristics. However, donor age has little influence on the outcome of islet transplantation.

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BETA CELL MASS IN CRYOPRESERVED ISLETS AFTER SYNGENEIC TRANSPLANTATION

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Cryopreservation remains the most practical method for long-term storage of islet tissue before islet transplantation (Tx). However, a higher number of cryopreserved (CR) islets, compared with non-cryopreserved (Non-CR) islets, must be transplanted to achieve normoglycemia. Aim: to examine whether the higher requirement of CR islets may be due to an increased beta cell mass loss after Tx. Materials and Methods : streptozocin-diabetic Lewis rats (blood glucose: 28.3± 0.7 mM) were transplanted with 1400 syngeneic islets (700 Non-CR and 700 CR under each of the kidney capsules). Islets were cryopreserved by the sequential addition of DMSO and a slow cooling rate (0.25°C/min from -7.5°C to -40°C).The grafts were harvested at 4 (group 1, n=7), 14 (group 2, n=7) and 56 (group 3, n=9) days after Tx. Beta cell mass was determined by point counting morphometry; beta cell replication was determined by bromodeoxyuridine incorporation and expressed as percentage of positive beta cells. Results: Blood glucose was 12.6± 2.0 mM in group 1, 9.7±2.2 mM in group 2 and 5.5±0.2 mM in group 3. Beta-cell mass was similar in CR and Non-CR islet grafts (group 1: 0.40±0.08 mg and 0.50±0.07 mg; group 2: 1.04±0.31 mg and 1.15±0.29 mg; group 3: 1.23±0.27 mg and 1.43±0.17 mg, respectively), and lower than the initially CR and Non-CR transplanted beta cell mass (2.52±0.16 mg and 2.98±0.20 mg, respectively)($p<0.01$). Beta cell replication was similar in CR and Non-CR islet grafts (group 1: 0.48±0.11% and 0.62±0.16%; group 2: 0.77±0.08% and 1.04±0.19%; group 3: 0.69±0.15% and 0.65±0.11%, respectively). Conclusion : beta cell mass and replication were similar in CR and Non-CR islets after Tx, suggesting that the requirement of a larger number of islets to restore normoglycemia is not explained by a higher loss of beta-cell mass in CR islets after Tx.

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INFLUENCE OF PORTAL-VENOUS VS. SYSTEMIC-VENOUS DRAINAGE AFTER PANCREAS-TRANSPLANTATION ON HYPERINSULINAEMIA

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Aims: Pancreas transplantation is usually performed with pancreas graft vein anastomosis to the iliacal veins (systemic-venous drainage). The circumvention of extraction by the liver may lead to hyperinsulinaemia, but its extent and potential influence on long term complications are controversial. **Materials and Methods:** Seven Type 1 diabetic patients (2 male / 5 female, 43 ± 7 y., BMI 21.4 ± 2.5 kg/m^2, HbA_{1c} 5.5 ± 0.4 %) studied 3 ± 2 months after combined pancreas and kidney transplantation by the portal-venous drainage technique were compared to eight patients (3 male / 5 female, 41 ± 7 y., BMI 21.1 ± 2.7 kg/m^2, HbA_{1c} 5.2 ± 0.8 %) studied 6 ± 4 months after transplantation with graft vein anastomosis to the iliac veins. Both patient groups were studied on two occasions in the morning after an overnight fast (a) with oral glucose (75 g), (b) with intravenous glucagon (1 mg). Blood was drawn for measurement of plasma glucose, insulin, C-peptide, proinsulin and free-fatty-acids. Statistics: RM-ANOVA, t-tests. **Results:** There were no significant differences in glucose excursions after oral glucose ($p = 0.27$). Insulin levels were significantly lower after portal-venous drainage compared to systemic-venous drainage, both in the basal state (5.4 ± 0.6 vs. 13.5 ± 2.7 mU/l, $p = 0.02$) and after stimulation with oral glucose ($p = 0.035$) or i.v. glucagon ($p = 0.029$). There were no significant differences for plasma concentrations of C-peptide (OGTT / i.v. glucagon; $p = 0.32$ / $p = 0.60$), proinsulin ($p = 0.26$ / $p = 0.60$) and free-fatty acids ($p = 0.99$ / $p = 0.31$). **Conclusions:** Pancreas transplantation with portal-venous drainage is a means for circumventing basal and post-stimulatory hyperinsulinaemia.

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CARDIAC AUTONOMIC NEUROPATHY AFTER KIDNEY AND PANCREAS TRANSPLANTATION

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Aims: Currently, pancreas transplantation represents the only method enabling the establishment of long-term normoglycemia in patients with type 1 diabetes. We have analysed the effect of kidney and pancreas transplantation (TxK+P) on the course of cardiac autonomic neuropathy in type 1 diabetic patients with kidney failure. Materials and Methods: Cardiac autonomic function was repeatedly tested in 19 patients (male/female ratio 8/11) before and from 6 months to 7 years after a successful TxK+P with permanent insulin independence. Autonomic function tests including beat-to-beat heart rate (R-R) variation at rest (MSSD), during deep breathing (I/E), lying to standing (R-R30/15, R-Rmax/min) and systolic blood pressure response to standing (ΔsBP) were performed and assessed as described by Ewing and Sundkvist with the use of an original program. Power spectral analysis of resting heart rate variability (PSA HRV) during short term recording (TotPo, PoLF, PoHF, PSDLF, PSDHF, PoLF/HF - total power, spectral power, power density and power ratio of low and high frequency components) based on Fast Fourier Transform (VariaPulse TF3, Sima Media Olomouc, Czech Republic) was also used in 7 patients. Pre- and post-transplant results were compared by the paired Wilcoxon test. Results: Abnormal results of most function tests indicative of autonomic damage were obtained in the pre-transplant period. Post-transplant, solely slight improvement in heart rate response to standing (pre-Tx/post-Tx, mean±SD: R-R30/15 0,98 ± 0,03/1,01 ± 0,03 $p<0,01$; R-Rmax/min 1,06 ± 0,1/1,11 ± 0,19 $p<0,06$) had occurred before the end of the follow-up. No change in parameters of PSA HRV has been noted during the follow-up period. Conclusions: Advanced cardiac autonomic neuropathy present in type 1 diabetic patients with kidney failure is probably not reversible even by long-term normoglycemia following successful pancreas transplantation.

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ROLE OF METFORMIN ON ISLET ENGRAFTMENT AFTER TRANSPLANTATION IN HUMANS.

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AIMS: Islet engraftment is a major problem of islet transplantation. A reduction of insulin resistence could improve islet engraftment. The aim of our study was to investigate the role of metformin on the islet function in humans. **MATERIALS AND METHODS:** 19 islet transplantations were performed, simultaneously or after kidney, in 16 IDDM patients (3 pts received the islet twice). The islets, separated with the automated method, were infused intraportally. 6 patients received metformin (1:5 g/day), pentoxifylline (1.2 g/day) and nicotinamide (1.5 g/day) (**Group 1**), while 13 patients did not received these drugs (**Group 2**). All patients were treated with the same immunosuppressive therapy (ATG/ALG –IMTIX- for induction; prednisone, cyclosporine, azathioprine or mycofenolate mofetyl for chronic treatment). Fasting C-Peptide (ng/ml), exogenous insulin requirement (% of the pre-tranplant doses), insulin independence and fIRI after Arginine infusion (AUC μU/60 min) were considered in order to assess the islet function during the first 6 months.

RESULTS	Group 1	Group 2
Fasting C Pep 1week (ng/ml)	2.83	1.47 *
Fasting C Pep 2w (ng/ml)	4.19	2.13*
Insulin requir 1w (% of pre tx doses)	75%	120%
Insulin requir 3w (% of pre tx doses)	52%	79%
Insulin requir 6Ms (% of pre tx doses)	2%	59%
Insulin free 6Ms	2/4 pts	2/12 pts
Arginine AUC 1Ms(uU/60 min)	1410	1115

* statistically different, t Student test

CONCLUSIONS: a reduction of insulin requirement and an increase of C peptide release is observed in patients treated with metformin, probably through an improvement of islet engraftment.

OP 18

Vascular Dysfunction: In Vitro Studies

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LIPOPROTEIN LIPASE STIMULATES HUMAN VASCULAR SMOOTH MUSCLE CELL GROWTH.

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Diabetes is a major risk factor for atherosclerosis. The overproduction of lipoprotein lipase (LPL) that we previously documented in mononuclear cells of type II diabetic patients may contribute to the acceleration of this vascular injury by promoting vascular smooth muscle cell (VSMC) growth. **Aims:** The present study was aimed at examining the effect of LPL on VSMC proliferation. **Materials and Methods:** Human VSMCs were isolated from saphenous veins and cultured for 24h in DMEM supplemented with 20% fetal bovine serum. Growth-arrested VSMCs were treated with the appropriate experimental agents for 24h, then incubated in the presence of [methyl-3H]-thymidine for an additional 48h period. **Results:** Incubation of growth-arrested human VSMCs with purified endotoxin-free bovine LPL (1 μg/ml), in the absence of any added exogenous lipoproteins, induced VSMC proliferation (192% over control values, $P < 0.01$). Addition of lipoproteins to the culture media did not further enhance LPL effect. Inactivation of LPL by guanidine hydrochloride or immunoneutralization of the lipase by the monoclonal 5D2 antibody did not suppress the LPL stimulatory effect on VSMC growth. On the contrary, preincubation of VSMCs with the specific protein kinase C inhibitors, calphostin C and chelerythrine, totally abolished LPL-induced VSMC proliferation. In LPL-treated VSMCs, a significant increase in PKC activity was observed. Treatment of VSMCs with heparinase III (1 U/ml) totally inhibited LPL-induced human VSMC proliferation. Exposure of growth-arrested VSMCs to conditioned media from unstimulated human monocyte-derived macrophages (MDMs) significantly induced VSMC proliferation (172 ± 19% over control values, $P<0.01$). While treatment of VSMCs with heparinase totally suppressed this proliferative effect, immunoneutralization of MDM conditioned media with antibodies against LPL or platelet-derived growth factor did not abolish the growth response of VSMCs to MDM supernatants. **Conclusion:** These data indicate that LPL exerts a direct stimulatory effect on VSMC proliferation. This effect may contribute to the accelerated atherosclerosis associated with type II diabetes.

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EFFECTS OF LONG CHAIN FATTY ACIDS ON ANTIOXIDANT DEFENCES IN PORCINE VASCULAR SMOOTH MUSCLE CELLS.

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Aims: Oxidative stress is a major factor causing diabetic vasculopathy. Both glucose and fatty acid metabolism are abnormal in diabetes. The aim of this project is to study how common long chain fatty acids (FAs) alter the response of vascular smooth muscle cells (VSMC) to glucose-induced oxidative stress. **Materials and Methods**: VSMC were cultured in 5 mM or 25 mM glucose and a mixture of 0.4mM palmitic (16:0) and 0.8mM oleic (18:1) acids (BSA as carrier). Activity of key antioxidants, glutathione (GSH), catalase, glutathione peroxidase (GPX), and superoxide dismutases (SODs) were measured. Malondialdehyde (MDA) and protein carbonyls (markers of lipid and protein peroxidation respectively) were analysed, as was oxidative DNA damage using the Comet assay. Levels of catalase mRNA were also measured. **Results**: GSH levels in 25mM glucose fell from 3.7±0.7 to 1.3±0.1 U/mg protein, $p<0.001$, indicating oxidative stress. FAs had no further effect on GSH. Catalase activity and mRNA levels were not altered by glucose alone. At 5mM glucose FAs caused no change in catalase mRNA but activity increased from 6.7±1.2 to 15.9±2.2 U/mg protein, $p<0.001$; at 25mM glucose, FAs caused a 44.4% increase in mRNA, $p<0.02$, while activity increased from 7.4±1.1 to 18.5±2.6 U/mg protein, $p<0.001$. Neither glucose nor FAs affected GPX, CuZnSOD, or MnSOD activity. MDA levels decreased in the presence of BSA and FAs, $p<0.04$, a result supported by decreasing trends in protein carbonyls and DNA damage. **Conclusions:** It is proposed that increased fatty acid metabolism is responsible for increased transcription and translation of catalase. The increased catalase activity in the presence of fatty acids may be responsible, at least in part, for reduced free radical damage to VSMC under these conditions.

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HIGH GLUCOSE EXPOSURE INDUCES GLUTATHIONE PEROXIDASE IN BOVINE RETINAL ENDOTHELIAL CELLS.

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Aims : The mechanisms by which hyperglycemia induces retinopathy are still poorly understood but are likely to be multiple. Nonreceptor mediated endothelial glucose signaling through either hyperosmolar or oxidative stress could contribute to this microvascular injury. **Materials and Methods** : In this study, we first determined glutathione peroxidase (Se-GPx) activity in native bovine retinal microvessels and cultured bovine retinal microvascular cells : endothelial cells (BREC) and pericytes (BRP). We further investigated the effects of hyperglycemia (up to 50 mM) on glutathione peroxidase (Se-GPx) in BREC by enzymatic assay and western blotting, and compared it to non-permeant osmolytes such as mannitol or NaCl. Cells were cultured chronically with or without agents during 2 passages (6 days on average). **Results** : Basal Se-GPx activity of pericytes in culture was very low contrasting with that found in BREC (1.1 ± 0.2 vs 22.0 ± 1.8 U/mg of proteins). In BRP, either Se-GPx, catalase or superoxide dismutase (SOD) were not modified by hyperglycemia or mannitol. For BREC, high glucose (25 mM or 50 mM) modified only Se-GPx activity (117 % of control at 50 mM glucose, $p<0.05$) but decreased it by 20 % ($p<0.05$) when compared to 50 mM mannitol. This increase was reproduced by NaCl (25 mM) (137 % of control, $p<0.05$), isoosmotic to mannitol. Induction of enzymatically active Se-GPx protein in BREC incubated with glucose (135 % of control, $p<0.05$), mannitol (156 %, $p<0.05$) or NaCl (166 %, $p<0.05$) was further confirmed by western blotting. In parallel, markers of lipid peroxidation such as thiobarbituric acid reactive substances (TBARS) or an isoprostane (8-epi-$PGF_{2\alpha}$) were not modified in BREC incubated with glucose or mannitol. **Conclusions** : These data indicate that (1) there is an induction of Se-GPx during hyperosmotic stress induced by glucose, mannitol and NaCl, which is specific of BREC and (2) this induction might represents an important endothelial antioxidant defense mechanism in response to osmotic stress.

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INCREASED RETINAL ENDOTHELIAL CELL PERMEABILITY INDUCED BY THE DIABETIC *MILIEU*: ROLE OF OXIDATIVE STRESS

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Vascular permeability was shown to be increased in tissues targets of diabetic complications early in the course of the disease. The increased permeability occurring at the retinal level may be involved in the pathogenesis of retinopathy by favoring the accumulation of circulating macromolecules and cells within the tissue. **Aim.** To investigate the role of oxidative stress in the increased retinal endothelial cell permeability, which we have previously shown to be induced by the diabetic *milieu*. **Materials and Methods.** Bovine retinal endothelial cells (BREC) were (a) either seeded on permeable membranes and exposed to media containing high glucose (HG, 30 mM) vs. normal glucose (NG, 10 mM) for 1 or 30 days or (b) grown on membranes previously coated with AGE-modified BSA vs. native BSA, ± various antioxidants. Monolayer permeability was assessed as transendothelial passage of ^{125}I-albumin over 5h. **Results.** Permeability was increased by incubation in HG vs. NG for 1 day (9.6±1.7% vs. 5.4±0.8% at 5h). These increases were reduced or virtually normalized by superoxide dismutase (SOD, 150U/ml; 6.3±0.7%), catalase (150U/ml; 8.1±1.3%), SOD + catalase (6.1±0.8%) and deferioxamine (5mM, 7.1±1.0%). Leakege of ^{125}I-albumin was also decreased dose-dependently by Vitamin E (6.8±1.0% with 100μM), but not by its acetylated or quinone forms, deprived of antioxidant activity. Likewise, the increases in permeability observed in BREC grown in HG for 30 days (8.1±0.9% vs. 5.1±0.3%) or on BSA-AGE vs. BSA (7.1±0.7% vs. 3.8±0.3%) were prevented or attenuated by these interventions. Under HG conditions, superoxide and lipoperoxide levels were significantly increased vs. NG. **Conclusions.** These results indicate that increased monolayer permeability induced by HG or BSA-AGE is mediated by the associated increase in oxygen free radical generation. Antioxidants might hence be helpful in the prevention or treatment of retinopathy.

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NITRIC OXIDE PRODUCTION AND HIGH GLUCOSE-ENHANCED RETINAL ENDOTHELIAL CELL MONOLAYER PERMEABILITY

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In previous studies, we have demonstrated that high glucose is capable of increasing retinal endothelial cell monolayer permeability, which is considered to be involved in the pathogenesis of diabetic retinopathy. **Aim.** To investigate the role of nitric oxide (NO) production in the increased retinal permeability induced by high glucose. **Materials and Methods.** Bovine retinal endothelial cells (BREC) were seeded on permeable membranes and exposed to media containing high glucose (HG, 30 mM) vs. normal glucose (NG, 10 mM) for 1 or 30 days ± agents inhibiting NO production/action or mimicking NO action. Monolayer permeability was assessed as transendothelial passage of ^{125}I-albumin over 5h. NO synthase (NOs) levels, endothelial isoform, were assessed by immunofluorescence and Western blot, whereas nitrite/nitrate (NO_2/NO_3) levels were measured by the Griess reaction. **Results.** ^{125}I-albumin leakage induced by exposure to HG vs. NG for 1 day (9.6±1.7% vs. 5.4±0.8% at 5h), but not to HG vs. NG for 30 days (8.1±0.9% vs. 5.1±0.3%), was reduced by the NOs inhibitors L-N-monomethylarginine (7.2±1.1%), aminoguanidine (7.1±2.0%) and methylguanidine (7.3±2.0%) and the NO-scavenger CPTIO (7.5±1.1%) at 1 mM concentration. HG-induced changes were further enhanced by the NO-donor sodium nitroprussiate (SNP, 1 mM) and reproduced by the NO and superoxide donor 3-morpholinosydnonimine (SIN-1, 1 mM) and their product peroxynitrite ($ONOO^-$, 40 μM). The effects of SNP and SIN-1 were prevented by CPTIO, superoxide dismutase, and uric acid, the latter attenuating also the permeability changes induced by $ONOO^-$. Under HG conditions, NOs expression and NO_2/NO_3 production were enhanced at day 1 and reduced thereafter. **Conclusions.** The short-term, but not the long-term, effects of HG on retinal endothelial permeability seem to be related to an increased activity of the endothelial NOs and consequent NO production which, in the presence of enhanced superoxide generation, results in increased peroxynitrite formation.

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FUNCTIONAL ASPECTS OF ANGIOTENSIN II RECEPTORS IN HUMAN GLOMERULAR ENDOTHELIAL CELLS (HGENC)

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Two major AII receptor subtypes (AT_1 and AT_2) have been identified. In adult kidney AT_1 predominates and accounts for most of the biological actions of AII; however 10% of AII receptors are AT_2. Which of the effects of AII in the kidney are actually transduced by AT_2 is currently not well understood. **Aims**: this study was carried out to identify AT_2 receptors in HGENC and to investigate a possible physiological role. **Materials and methods**: for binding experiments HGENC at second (-2p) and ninth passage (-9p) have been grown at confluence and serum deprived in 24-well plates for 24 hr and then incubated in presence of [^{125}I](Sar-1, Ile-8)AII with increasing concentrations of AII, DUP753 (AT_1 antagonist) and PD123177 (AT_2 antagonist). For DNA synthesis HGENC were plated in 24-well plates, maintained in serum free for 24 hr and then treated with stimuli. HGENC were then incubated with [^{3}H]thymidine during the last 24 hr of stimulation. **Results**: Scatchard analysis in HGENC-2p showed the presence of one class of binding sites with an apparent Kd 1.01 nM and a Bmax of 5.2 fmoles/1E5 cells. The pharmacological characterization of AII receptors revealed that AII and PD123 compete for the AII binding with different affinities: 2.44 nM and 81nM respectively; DUP753 was unable to displace the tracer at concentrations up to 10μM. Instead, binding studies on HGENC-9p demonstrated complex competition curves compatible with the presence of both AT_1 and AT_2 receptors. The parameters of these binding sites did not depend on the cellular density. We next studied the AT_2 actions on HGENC: the addition of AII (100nM) to HGENC-2p determined a slightly significant inhibitory action which was not influenced by DUP753; in contrast, pretreatment of HGENC with PD123 totally prevented the AII antimitogenic effect. In later passages (HGENC-9p) DUP753 pretreatment reduced AII thymidine uptake, while PD123 determined an AII mitogenic stimulation. This effect was abolished when HGENC were pretreated with DUP753 in addition to PD123. Finally, AII was able to stimulate the release of PDGF only in HGENC-9p and this effect was counteracted by the presence of DUP753. **Conclusions**: i) HGENC present AII receptor subtypes depending on the cell passage, ii) AT_2 seem to have a predominant inhibitory effect on HGENC growth, iii) AT_1 could promote cell growth, probably also modulating the PDGF release.

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REGULATION OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-3, -4 AND -5 EXPRESSION IN ENDOTHELIAL CELLS.

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Aims: To investigate the effect of growth factors associated with diabetic complications on the expression of insulin-like growth factor binding proteins (IGFBPs) in endothelial cells. **Materials and Methods:** Cultured endothelial cells from bovine aorta were stimulated with vascular endothelial growth factor (VEGF), transforming growth factor-ß1 (TGF-ß1), IGF-I, insulin or angiotensin II for 18 hours. Gene expression of IGFBPs was measured by solution hybridization. Values are given as means±SD and statistical comparisons are made according to ANOVA Scheffé F-test. **Results:** IGFBP-3, -4 and -5 mRNA was abundantly expressed by the endothelial cells while IGFBP-1 was not detected and IGFBP-2 and -6 mRNA expression was at the limit of detection. VEGF inhibited the expression of IGFBP-3 mRNA (45±6 % of control; P<0.01), had no effect on IGFBP-4 and strongly enhanced IGFBP-5 expression (251±68 % of control; p<0.01). TGF-ß1 inhibited IGFBP-3 mRNA expression (38±9 % of control; p<0.001), as also described earlier. In addition we showed that IGFBP-4 mRNA was inhibited by TGF-ß1 (58±17 % of control; p<0.05) and that IGFBP-5 was not significantly altered although a tendency to inhibition was seen (68±6 % of control). IGF-I, insulin or angiotensin II did not have any effect on IGFBP mRNA expression. **Conclusions:** VEGF and TGF-ß1, both associated with diabetic complications, differentially regulate gene expression of IGFBP-3, -4 and -5 in bovine aortic endothelial cells.

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Granule Traffic in the Pancreatic β-Cell

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LOCALISATION AND FUNCTIONAL ACTIVITY OF A SOLUBLE SNAP-25 MUTANT IN INSULIN SECRETING HIT CELLS

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The t-SNARE SNAP-25 is a palmitoylated protein associated with the plasma membrane. It is essential for granule docking/fusion in regulated insulin secretion. **Aims:** To investigate the cellular localisation and functional activity in HIT cells of SNAP-25 mutated in one or two of the four putative palmitoylated cysteines. **Materials and Methods:** A GFP-(Green Fluorescent Protein)-SNAP-25 fusion protein, resistant to cleavage by Botulinum neurotoxin E (BoNT/E), was used to produce GFP-SNAP-25 fusion proteins mutated at Cys^{85} or $Cys^{85,88}$. Proteins were expressed in HIT cells and cellular localisation examined by fluorescence microscopy or cell fractionation (membrane vs cytosol) followed by Western Blotting. The ability of the toxin-resistant GFP-SNAP-25 and the Cys mutants to promote insulin secretion was tested in HIT cells following inactivation of endogenous SNAP-25 by BoNT/E. **Results:** The toxin-resistant GFP-SNAP-25 fusion protein was localised at the plasma membrane and reconstituted insulin secretion from BoNT/E treated cells as efficiently as toxin-resistant SNAP-25, which allowed to exclude artefacts due to GFP. Cell fractionation of HIT cells transfected with GFP or toxin-resistant GFP-SNAP-25 (as controls for cytosolic and membrane localisation, respectively) or the toxin-resistant Cys mutants showed that the single (Cys^{85}/Ala) mutant remained partially on the membrane whereas the double mutant ($Cys^{85,88}$/Ala) was totally cytosolic; fluorescence microscopy confirmed these results. At the functional level the toxin-resistant GFP-SNAP-25 single Cys mutant (Cys^{85}/Ala), although partially cytosolic, reconstituted completely insulin secretion when expressed in HIT cells exposed to BoNT/E. Despite being completely cytosolic, the double Cys mutant ($Cys^{85,88}$/Ala) retained some functional activity. **Conclusions:** These results suggest that Cys residues 85 and 88 are necessary for membrane association of SNAP-25 in insulin secreting cells and that the functional activity of SNAP-25 is not completely abolished when it is not membrane associated.

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Comigration of Synapsin1 und CaM Kin II δ2 with Insulin in INS-1

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Background: Numerous data indicate a role of Calcium/Calmodulin-dependent protein kinase II (CaM Kin II) in insulin secretion but the mechanism of its action is poorly understood. The phosphorylation of Synapsin1 by CaM Kin II leading to debundling of actin fibers and release of actin bound granules to the readily releasable pool is one conceivable feature comparable to its function in neurosecretion. **Methods:** Subcellular fractions of INS-1 insulinoma cells were made by sucrose density gradient centrifugation and by nycodenz gradient centrifugation. Analysis of fractions was performed by protein measurement, refractometry, insulin-RIA, arylsulfatase activity assay and immunoblotting with laserdensitometric quantification. **Results:** A protein band of the aspected size of 85 kDa was detected using a Synapsin1-directed antibody in immunoblots of INS-1. Synapsin1 paralleled CaM Kin II δ2 and Insulin in fractions of the sucrose density gradient of INS-1. On the other hand markers for endoplasmatic reticulum, plasma membrane and synaptic like vesicles had different patterns of subcellular distribution. Concentration of insulin by nycodenz density centrifugation resulted in the enrichment of both CaM Kin II δ2 and Synapsin1. **Conclusion:** A protein of 85 kDa supposed to be Synapsin1 was detected in immunblot of INS-1. Comigration of CaM Kin II δ2 and Synapsin1 with insulin strongly supports the hypothesis of their interaction during insulin release.

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TRANSLOCATION OF MYOSIN LIGHT CHAIN KINASE MAY CONTRIBUTE TO PROTEIN KINASE C-POTENTIATION OF INSULIN RELEASE.

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Aim: This study was undertaken to investigate underlying mechanism for protein kinase C activation of insulin release. **Materials and Methods:** All experiments were carried out using MIN6 beta-cells. Intracellular movement of the insulin granules was visualized by phase-contrast microscopy and an image analyzer. Distribution of the insulin granules and myosin light chain kinase (MLCK) was examined by immunofluorescence and immunoelectron microscopies. Insulin secretion was measured by radioimmunoassay. **Results:** Activation of protein kinase C by 12-*O*-tetradecanoyl-phorbol 13-acetate (TPA) caused a shift of insulin granules to the cell periphery, which was not reproduced by forskolin, the adenylate cyclase activator. In contrast, forskolin, but not TPA, activated the granule traffic. We found that MLCK, which regulates intracellular movement of insulin granules, was also translocated to the plasma membrane under activation of protein kinase C. Morphological studies demonstrated that considerable parts of MLCK immunoreactivity were co-localized with the insulin granules. Co-activation of protein kinases A and C resulted in a further increase of the number of peripheral granules, and in strong potentiation of insulin release. **Conclusion:** Protein kinases A and C may act on granule traffic and docking/priming, respectively, in the secretory cascade upstream exocytosis, and translocation of MLCK under protein kinase C activation may activate granule traffic in the vicinity of the plasma membrane and increase the number of readily-releasable granules.

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POSSIBLE ROLES OF ACTIN FIBERS IN INTRACELLULAR DISTRIBUTION OF INSULIN GRANULES.

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Aim: The aim of this study was to investigate the motor protein/cytoskeleton system relevant for intracellular traffic of insulin granules in the pancreatic beta-cell. **Materials and Methods:** MIN6 beta-cells were used in whole experiments. Insulin granule traffic in living cells was observed by phase-contrast microscopy and intracellular distribution of F-actin and insulin were determined by FITC-phalloidin and anti-insulin antibody, respectively. For immunoblotting, MIN6 extracts were fractionated by sucrose-gradient ultracentrifugation, and insulin secretion was determined by radioimmunoassay. **Results:** Bioimaging of the intracellular movement of the insulin granules revealed that the granule movement was transiently activated by depolymerization of F-actin with mycalolide B, which was not reproduced by treatment with colchicine. Western blotting of sucrose-gradient fractions of MIN6 extracts demonstrated that myosin light chain exists in the insulin-rich fractions, but not in synaptophysin-rich ones (small density granules). In MIN6 cells, F-actin did not form evident barrier structure beneath plasma membranes. However, mycalolide B treatment of MIN6 cells caused marked translocation of the insulin ranules in the vicinity of the plasma membrane and resulted in a rapid increase of insulin output. **Conclusion:** We suggest that the bata-cell granules are associated with myosin as a motor protein, and that actin fibers may play dual roles in the insulin secretory machinery (cable for traffic and hindrance to granule docking).

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ROLE FOR $[Ca^{2+}]$ MICRODOMAINS IN GLUCOSE-INDUCED MOVEMENT OF GFP-TAGGED SECRETORY GRANULES.

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Aims. We have previously demonstrated that enhanced green fluorescent protein (EGFP) can be targeted to insulin secretory granules with high specificity after expression of chimaeric cDNA encoding a phogrin.EGFP fusion protein. Elevated glucose concentrations prompt granule movement, an effect mimicked by increases in intracellular $[Ca^{2+}]$. The aim of this study was to monitor the effect on secretory granule mobility of intracellular modulators of insulin secretion, including Ca^{2+} and cAMP, introduced into the cell cytosol through the patch pipette. **Materials and Methods.** Combined laser-scanning confocal microscopy was performed with a Leica DM/IRBI inverted microscope (488 nm excitation, 510 nm emission), and standard electrophysiology equipment. **Results.** Under voltage clamp (-70 mV), dialysis of single INS-1 cells (passage # 90 – 100) with Mg.ATP (10 mM), cAMP (0.1 mM), and EGTA-buffered Ca^{2+} (2 μM) had little effect on secretory granule movement. However, further increases in intracellular $[Ca^{2+}]$ to > 100 μM, achieved by cell hyperpolorization, provoked the movement of vesicles towards the plasma membrane (visualised with the hydrophilic dye, FM4-64). **Conclusions.** Combined electrophysiology and dynamic EGFP imaging suggest that secretory vesicle movement requires concentrations of Ca^{2+} well in excess of those in the bulk cytosol, indicative of high $[Ca^{2+}]$ microdomains close to mobile granules.

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SPONTANEOUS GRANULAR LOCALIZATION AND REGULATED SECRETION OF GREEN FLUORESCENT PROTEIN IN INS CELLS.

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Aims: To follow intracellular trafficking and subcellular localization of proinsulin in living cells using Green Fluorescent Protein (GFP) fluorescence as a marker. **Materials and Methods:** A preproinsulin-GFP fusion protein (PPI-GFP) was expressed by transfection in INS-1 cells. The interpretation of experiments using GFP-fusion proteins is dependent upon the assumption that the localization of GFP is determined by the protein to which it is fused. A fusion protein consisting of signal peptide-GFP (Sp-GFP) but devoid of any proinsulin sequence was therefore used as a control. Cells were examined by confocal and electron microscopy. Release of GFP was measured by Western blot and of insulin by radioimmunoassay. **Results:** By confocal microscopy, fluorescence in PPI-GFP cells was compatible with localization of GFP in granules (with GFP fluorescence and insulin immunofluorescence superimposed). Unexpectedly, the same pattern was observed in Sp-GFP cells. The spontaneous granular localization of GFP independent of any proinsulin sequence in Sp-GFP cells was established by high resolution (EM) immunocytochemistry. In keeping with its granular compartmentalization, GFP secretion from INS cells expressing Sp-GFP was stimulated 8-10 fold by secretagogues and similar to insulin, with limited basal secretion (GFP: 4.5±0.3 % cell content/h vs insulin: 4.8±1.3). Such was not the case when a protein secreted by the constitutive pathway, secretory alkaline phosphatase (SEAP), was expressed in INS cells (<2-fold stimulation; basal secretion 17±2.6%/h), confirming that the constitutive and regulated pathways do coexist in INS cells. **Conclusions:** In INS cells, GFP (but not SEAP) is unexpectedly recognized as a regulated secretory protein and stored along with insulin in granules. This raises questions about the specificity and mechanism of this sorting process and, in a more general context, concerns about the validity of GFP as a passive indicator of secretory protein localization.

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INOSITOL HEXAKISPHOSPHATE STIMULATES DYNAMIN-1 MEDIATED ENDOCYTOSIS IN PANCREATIC B-CELLS

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Inositol hexakisphosphate ($InsP_6$) is under metabolic control and serves as a signal in the pancreatic B-cell stimulus-secretion coupling by increasing Ca^{2+}-channel activity and insulin exocytosis. We have explored the effects of $InsP_6$ on endocytosis in mouse pancreatic B-cells using the standard whole-cell configuration of the patch-clamp technique and capacitance measurements. $InsP_6$ elicited endocytosis in a Ca^{2+}-EGTA buffer with a free Ca^{2+} concentration of 54 nM without a concomitant stimulation of exocytosis. The effect was concentration dependent with a half-maximal stimulatory effect at 10.2 μM. Using the membrane dye FM1-43 with quantitative fluorescence microscopy we found that $InsP_6$ stimulated endocytosis in the presence of both low (30 nM) and high cytoplasmic Ca^{2+} concentrations (10 μM). This effect was mediated by activation of dynamin I and was not observed in cells treated with antisense oligonucleotides against dynamin I but not dynamin II. Western blot analysis with specific antibodies for dynamin I and II demonstrated the expression of both forms of dynamin in the B-cell. Dynamin I is a high-affinity substrate for the protein phosphatase (PP2B) calcineurin and endocytosis was abolished by the specific calcineurin inhibitors deltamethrin and cyclosporin A. By contrast, okadaic acid (an inhibitor of type 1, 2A and 3 phosphatases) failed to affect endocytosis. Dynamin I is also a substrate for protein kinase C (PKC) and the stimulatory effect of $InsP_6$ on endocytosis was antagonised by the PKC inhibitors calphostin C and bisindolylmaleimide but not by the protein kinase A inhibitor Rp-cAMPS. These data suggest that endocytosis may involve a time-dependent dephosphorylation and rephosphorylation of dynamin I by sequential activation of the serine-threonine protein phosphatase calcineurin and protein kinase C. This suggests that $InsP_6$ serves as a physiological signal regulating vesicular trafficking in insulin secreting cells.

OP 20

Psychosocial Aspects

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DETERMINANTS OF THE QUALITY OF LIFE OF PATIENTS WITH TYPE 1 DIABETES IN TURKEY

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Aims: The concept of Quality of Life (QOL), is understood as a multidimensional construct made up of physiological, psychological and social aspects.In this study, we examined the overall QOL and its possible relationships with sociodemographic, diabetes-specific and metabolic control parameters as well as presence of diabetic complications in patients with Type 1 diabetes. **Materials and methods:** A cohort of 300 patients with Type 1 diabetes (mean age 24.4±9.6 years, 144 female, 156 male, diabetes duration 12.6±4.8) were included in this study.The evaluation was made based on "Diabetes Quality of Life Measure" (DQOL) questionnaire, after standardization and validation to Turkish population characteristics. The questionnaire composed a total of 46 items investigating four different subscales, satisfaction (15), impact (20), social/vocational worry (7) and diabetes worry (4).One way anova and student-t test were used to detect the specific contribution of each variable to DQOL.**Results:** Mean score was 73.58±12.6 in females and 73.62±13.4 in males.Our study suggested that DQOL strongly correlated with diabetes duration ($p<0,05$), economical level ($p<0.001$), general education ($p<0,01$), diabetes education ($p<0,001$), adaptation to diabetes therapy ($p<0,001$), adaptation to dietary therapy ($p(0,001$), frequency of regular exercise ($p<0,01$), frequency of follow-up visits ($p<0,001$), frequency of home-monitoring ($p<0,001$), habituals (smoking and alcohol) ($p<0,001$), FBG ($p<0,05$), HbA1c ($p<0,01$),BMI ($p<0,05$), frequency of hypoglycemia ($p<0,05$), and presence of chronic complications ($p<0,001$).On the other hand sex, mode of therapy (conventional versus intensive insulin therapy), house environment, family history of diabetes, frequency of hypoglycemia, did not appear to be interfered with DQOL scores. **Conclusions**:The results indicated that QOL in our Type 1 diabetic population is moderate. Education and good metabolic control along with self monitoring facilities will not only postponed the development of devastating complications, but will certainly improve the overall QOL of these patients.

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THE IMPACT OF DIABETIC COMPLICATIONS ON THE QUALITY OF LIFE OF PATIENTS WITH TYPE 2 DIABETES

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Aims: This study investigated the impact of long-term diabetic complications on the quality of life (QoL) of patients with type 2 diabetes.

Methods: A total of 1230 patients from three clinical trials completed the SF-36 QoL questionnaire at baseline. Demographic details, body mass index (BMI), blood glucose (HbA_{1c} and FSG), and the presence and severity of nine specified diabetic complications were also collected. To establish the key determinants of QoL, each scale of the SF-36 was analysed by multiple regression analysis using the above factors as explanatory variables. Repeated stepwise backwards elimination was used to remove the least significant factors from the model until all remaining factors were significant at the 10% level.

Results: The mean (SD) age of the patients was 61.1 years (10.8); mean FSG was 11.1 mmol/l (3.0), and mean HbA_{1c} was 8.0% (1.4). Fifty-seven percent of patients were male. The most prevalent diabetic complications were hypertension (46% patients), peripheral sensory neuropathy (PSN; 12%), coronary artery disease (CAD; 8%) and peripheral vascular disease (PVD; 7%). Most (73%) of the complications reported were assessed to be 'mild', 22% 'moderate' and 5% 'severe'. Any presence of PSN was associated with significantly lower scores (ie worse quality of life) in the role–emotional, bodily pain and mental health scales; CAD with significant reduction of all SF-36 scales except role–emotional and mental health; and PVD with significantly lower general health, physical and social functioning scales. Hypertension did not have an independent effect on QoL.

Conclusion: Many aspects of QoL are adversely affected by diabetic complications, in particular by CAD. The presence of even mild diabetic complications has a significant impact on patients' quality of life.

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MEDIATORS OF QUALITY OF LIFE DURING STAGED DIABETES MANAGEMENT VS. USUAL CARE IN TYPE 2 DIABETES

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Aims: The positive effects of oral hypoglycemic therapy on quality of life (QOL) in type 2 diabetes are mediated primarily by improvements in hyperglycemic symptoms. However, other management practices such as specialty care, diabetes nurse education or treatment algorithms might result in QOL improvements independently mediated by other mechanisms such as improved psychological functioning.

Materials and Methods: The impact of Staged Diabetes Management (SDM) using nurse educators supervised by an endocrinologist versus Usual Care (UC) by a primary care physician (PCP) on QOL and glycemic control was evaluated in 106 SDM patients and 77 UC patients during a 12-month, unblinded, clinical trial.

Results: Patients were 48% male and 59% Caucasian; 80% completed high school and 66% were currently married (p=ns between groups). At baseline, the SDM group was slightly younger (60±1.1 vs. 65±1.2 yrs, p<0.01) with somewhat higher HbA_{1c} levels (8.5±0.2 vs. 8.1±0.2%, p=0.07). HbA_{1c} decreases from baseline were greater for SDM at both six (1.4±0.1 vs. 0.7±0.2%, p=0.002) and twelve (1.1±0.1 vs. 0.4±0.2%, p=0.004) months. No QOL differences were present at baseline. However, significant differences controlling for age were found for Overall QOL which improved at six months by +0.66 standard deviation (SD) units in the SDM group and worsened by -0.22 SD units in the UC group, p<0.001. QOL remained significantly different at final visit (-0.25 vs. +0.46, p=0.01). While all QOL changes favored SDM, those contributing the largest differences (SDM/UC) were symptom distress (+0.81/-0.31, p=0.002), health status (+0.50/-0.21, p=0.02), health perceptions (+0.54/-0.07, p=0.03), vitality (+0.41/-0.17, p=0.03), symptom interference (+0.06/-0.50, p=0.03), psychological well being (+0.26/-0.26, p=0.04), emotional ties (+0.18/-0.28, p=0.03) and positive affect (+0.19/-0.20, p=0.04). Stepwise regression analysis revealed that both symptom distress (p=0.03) and psychological distress (p=0.02) were significantly affected by changes in HbA_{1c} independently from treatment assignment.

Conclusions: While improved glycemic control mediates QOL changes primarily through reductions in physical and psychological distress, SDM provides an independent benefit through improved emotional and psychological well being.

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COGNITIVE BEHAVIOURAL GROUP TRAINING FOR TYPE I PATIENTS IN POOR CONTROL: EFFECTS ON GLYCAEMIC CONTROL AND WELL-BEING.

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Aims: A substantial number of type I DM patients does not succeed in reaching good glycaemic control, and are thus at increased risk of developing complications. Next to optimal medical treatment and education, the need for psychosocial interventions to assist these patients to cope more effectively with their selfmanagement is generally acknowledged. The aim of this pilot study was to develop and evaluate a short Cognitive Behavioural Group Training (CBGT) for type I DM patients in poor control. Methods: The effects of CBGT on selfcare-behaviour, diabetes-related distress (PAID, 0-100), emotional well-being (WBQ, 0-36), and glycaemic control (HbA1c, ref. 4.3-6.1%) were evaluated in a prospective study with 24 type I DM patients in poor control (HbA1c>8.5%), (mean age 35.17 ± 11.13 yrs; 9 male/15 female; mean duration of diabetes 17.62 ± 9.35 yrs; Mean HbA1c 9.2% ± 1.2), with no serious medical or psychiatric co-morbidity. CBGT consists of 4 weekly sessions delivered by a psychologist and diabetes educator, with cognitive restructuring and stress-management as main components. Results: Results for the first 3 groups (n=16) at 3 months follow-up show a decrease in diabetes-related distress, (mean PAID-score drop: 45.08 to 35.95, t=-3.46, p<0.001), and an increase in positive well-being (mean PosWBQ-score 6.94 to 7.63, t=-2.42, p<0.05). Changes in self-care were highly variable, as expected given the high variability at baseline. Glycaemic control tended to improve (mean HbA1c drop: 9.57 to 8.86%, t=-1.83, p=0.088), with greatest benefits for those in worst control. Formative evaluations show CBGT was feasible in terms of protocol integrity and attendance, and highly appreciated by participants. Conclusion: This pilot study demonstrates that CBGT, a short psycho-educational intervention, is feasible and suggests that it assists type I DM patients with a history of persistent poor control to improve their glycaemic control and quality of life.

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PSYCHOLOGICAL COMORBIDITY IN DIABETIC PATIENTS FEARFUL OF SELF-INJECTING AND/OR SELF-TESTING

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Aims: The prevalence of both fear of injecting and fear of self-testing is estimated to be ± 1-2 % among insulin-treated diabetic patients. One of the aims of this ongoing study is to characterize patients who are fearful of self-injecting/self-testing in terms of depression, psychopathology and other fears/phobias. **Materials and methods:** In a survey among 1484 members from the Dutch Diabetic Association, data were gathered on a number of questionnaires, including the Diabetes Fear of Injecting and Self-testing Questionnaire (D-FISQ). Highscorers on the D-FISQ (n=119) were selected using a cut-off score ($\geq 95^{th}$ percentile) on one or both of the subscales (resp. fear of self-injecting [FSI] & fear of self-testing [FST]). Subjects were requested to complete questionnaires providing information on psycho-pathology (Symptom Checklist; SCL-90), depression (Becks Depression Inventory; BDI) and fears/phobias (Fear Survey Schedule; FSS-III-R). The scores of the subjects on these questionnaires were compared to normgroups. High scores are defined as mean + 2sd (expected prevalence in a population = 5%). **Results:** Respons was 70% (n=83); 44% male, 52 % type 1, mean age 47.0 (± 15.9) years, mean DM duration 13.9 (± 9.7) years, mean insulin use 12.7 (±10.0) years. In this sample, 11% reported high scores on depression (BDI). 13% scored high on anxiety (SCL-90) and 11% on hostility (SCL-90). The FSS-III-R scores demonstrated a relative high prevalence of social fear (14%), agoraphobia (35%), fear of animals (18%), and fear of aggressive/sexual scenes (31%); 24% scored highly on fear of blood/injections/ wounds. **Conclusions:** Prevalence of psychological comorbidity -especially fears and phobias- is high in this group of patients fearful of of self-injecting/self-testing. This underscores the complexity of the psychological problems of these patients and the need to tailor treatment to the individual patient.

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THE ASSOCIATION OF METABOLIC CONTROL AND QUALITY OF LIFE WITH SOCIAL STATUS AND ETHNIC MINORITY ORIGIN FOR ADOLESCENTS WITH DIABETES.

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Aims: Quality of life (QOL) of child and family was assessed using self-rating questionnaires on 2,077 adolescents with diabetes (boys 1,073, girls 1,004), median age 14.0 years (range 10-18 years), involving 17 countries including Europe, Japan and North America. HbA1c was analysed centrally and clinical data was collected in order to study the relationship of metabolic control and QOL with social status and ethnic minority origin. **Materials and Methods:** Adolescents completed the Diabetes Quality of Life Questionnaire, DQOL (impact, worries, satisfaction and health perception); and family burden was assessed by a multidimensional questionnaire to parents and health professionals. Regarding social status, parents were asked whether there were 1 or 2 parents at home, and if the parents were employed. The health professional was asked if the adolescent belonged to an ethnic minority or an immigrant group. **Results:** HbA1c was unrelated to social status (p=0.76), but was significantly higher in ethnic minorities (p=0.02). The QOL was positively related to social status (p=0.0006); and particularly to impact of diabetes (p=0.002) and satisfaction with life (p=0.003), but also to worries (p=0.05), health perception (p=0.11); and more modestly to family burden as perceived by parent (p=0.06), and by health professional (p=0.09). QOL for adolescents differed by ethnic status regarding impact of diabetes (p=0.03), but not worries (p=0.23), satisfaction (p=0.10), health perception (p=0.70), or family burden as perceived by parent (p=0.46), or health professional (p=0.87). Where different, QOL was lower for adolescents of minority status. **Conclusions:** Adolescents from families of lower social status and ethnic minority groups have a poorer QOL particularly as perceived by adolescents themselves, and in addition, ethnic minorities have a poorer HbA1c. Both groups require greater input of medical and social resources.

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PREDICTION OF CHILDREN'S ADJUSTMENT TO DIABETES

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Aims: To predict psychological adjustment and metabolic control at 5 years disease duration from assessments of affective reactions and psychological factors during the first year after onset of type 1 diabetes. **Materials and methods:** Children (n=67), 0-14 years of age were recruited for a longitudinal study immediately after onset of diabetes. Hospital staff assessed affective reactions to diabetes in all children, older children also assessed themselves. At 9 months children were assessed for psychological functioning (locus of control, clinical behavior, emotional well-being) and adjustment to diabetes. After 5 years diabetes duration, adjustment to diabetes was assessed by two questionnaires on adaptation and by depressive symptoms. The data was analyzed by multiple regression analysis with stepwise entering of possible predictor variables. **Results:** Affective reactions of increased anger and injections anxiety, but decreased general anxiety assessed by the child, predicted negative attitude to diabetes with 76% of the variance explained ($p=0.001$). Injection anxiety predicted overall adaptation to diabetes (28%; $p=0.037$). More anger assessed by the child predicted more symptoms of depression five years later (41%; $p=0.008$). When the staff assessed increased protest and more injection anxiety, the child expressed more negative attitudes (12%; $p=0.021$) more emotional difficulties (15%; $p=0.011$) and less good quality of life (9%; $p=0.041$) five years later. A more external locus of control predicted more emotional difficulties at 5-year follow-up (13%; $p=0.013$). Problems in adjustment at 9 months predicted adaptation problems at 5 year (16%; $p=0.022$). Major behavioral problems in clinical rating was associated to more anxiety at 5 year (38%; $p<0.001$). Metabolic control (Hemoglobin A1c) at five years diabetes duration was mainly explained by earlier metabolic control, but the result also indicated that a more internal locus of control as well as a higher score in the clinical rating could affect metabolic control. **Conclusion:** Early affective reactions, locus of control and adjustment to diabetes can predict psychological adjustment 5 years later. It should be of concern to early identify and treat patients with strong affective reactions and adjustment difficulties to reduce or prevent future adjustment problems.

OP 21
Stimulation of Insulin Secretion

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EFFECT OF NUTRIENT INGESTION ON GLUCAGON-LIKE PEPTIDE 1 (7-36 AMIDE) SECRETION IN TYPE 1 AND TYPE 2 DIABETES.

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Postprandial release of Glucagon-like Peptide 1 (GLP-1) 7-36 amide from intestinal L-cells strongly stimulates insulin secretion, contributing to the regulation of glucose homeostasis in healthy subjects. In pharmacological concentrations, GLP-1 is able to raise insulin and to low plasma glucagon levels also in type 2 diabetic patients, nearly normalizing both fasting and postprandial hyperglycaemia. Owing to its antidiabetogenic effects, the peptide reduces meal-related insulin requirement in type 1 diabetic patients as well, suggesting that GLP-1 may have therapeutic implications in both type 2 and type 1 diabetes treatment. Taken together these observations indicate that in conditions of hyperglycaemia an impairment of the entero-insular axis exists. **Aims**: to further assess this metabolic disorder evaluating the GLP-1 response to nutrient ingestion in type 1 and type 2 diabetes. **Methods**: 16 type 1 (age 40.5±14 yr, duration of disease 16±13.5 yr, HbA_1C 7.8±1.5%), 14 type 2 diabetic patients (age 56.5±13 yr, known duration of disease 8.6±7.7 yr, HbA_1C 8.1±1.8%) and 10 matched controls were studied. In postabsorptive state a standard mixed breakfast (230 Kcal: carbohydrates=60%, lipids=22%, proteins=18%) was administrated to all subjects and blood samples were collected at: 0,30,60,120,180 min. for plasma glucose, insulin, C-peptide, glucagon and GLP-1 determination. **Results**: in normal subjects the meal induced a significant increase of GLP-1 at 60 min ($p<0.05$), returning towards fasting levels in the following two hours. In type 1 diabetics basal GLP-1 values were similar to controls (106.5±6 vs 97.3±12 pg/ml), but the peptide response to the meal resulted significantly different ($p<0.01$), failing meal ingestion to increase postprandial GLP-1 concentrations, which even decreased during the test. A similar pattern in both basal and postprandial GLP-1 secretion was observed in the type 2 diabetic patients examined ($p<0.01$). No significant variations of plasma glucagon occurred following meal ingestion in both groups of patients examined. Statistical evaluation of the results was performed by analysis of variance. **Conclusions**: in spite of normal basal GLP-1 secretion, intestinal L-cell responsivity seems to be lost in diabetes, both in the condition of insulin deficiency and insulin resistance. It may be postulated that the continuous GLP-1 stimulation, secondary to chronic hyperglycaemia, may induce a progressive desensitization of L-cells with consequent peptide failure response to specific stimulation.

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THE EFFECT OF EXOGENOUS GLP-1 ON THE GLUCOSE MEDIATED INSULIN SECRETION: A DOSE-RESPONSE STUDY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND CONTROL SUBJECTS.

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Glucagon-like-peptide-1 (GLP-1) (7-36) amide is an intestinally derived hormone that stimulates insulin secretion both *in vivo* and *in vitro* including patients with Type 2 diabetes mellitus (DM). Being a future treatment for Type 2 diabetes, the optimal dosage and the effect on the glucose mediated insulin secretion needed to be established. We therefore performed dose-response curves in 7 patients with DM, fasting plasma glucose (FPG) ~7-9 mmol/l and 7 matched control subjects (CNT), FPG ~4.0-5.5 mmol/l. GLP-1 was infused intravenously on three separate occasions at concentrations of 0.5, 1.0 and 2.0 pmol/kg/min and saline infused on a fourth occasion as control, all in randomised order. During all four experiments, the glucose levels were stepwise clamped between 6-20 mmol/l using a graded intravenous glucose infusion. To explore the dose-relationship between glucose and insulin secretion rates (ISR), we calculated ISR by the use of peripheral plasma C-peptide profiles and the Deconvolution Method by Eaton/Polonsky, modified to population based parameters for C-peptide kinetics. The higher the GLP-1 infusion the more upward and leftward shifted the glucose- and ISR response curves became. This was observed for both DM and CNT subjects. The GLP-1 infusions resulted in significant elevations of the maximal ISR (MISR) pmol/kg/min, seen as a 3-fold increase in DM patients and a 2-fold increase in CNT subjects during the 2.0 pmol/kg/min GLP-1 infusion compared to the saline infusion (Table). The β-cell responsiveness to changes in glucose (ISR/FPG) also increased significantly (Table).

	DM	CNT		DM	CNT
Saline Infusion :			GLP-1 (1.0 mol/kg/min):		
MISR	4.2	13.0	MISR	10.4	21.6
β cell responsiveness	0.2	0.6	β cell responsiveness	0.7	3.0
GLP-1(0.5 pmol/kg/min):			GLP-1 (2.0 pmol/kg/min):		
MISR	9.7	20.3	MISR	12.8	25.2
β cell responsiveness	0.6	2.0	β cell responsiveness	1.3	6.7

We conclude that GLP-1 significantly enhances the β cell responsiveness to glucose as well as maximal secretory capacity.

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GLP-1 SECRETION IS DECREASED IN NIDDM PATIENTS COMPARED TO MATCHED CONTROL SUBJECTS WITH NORMAL GLUCOSE TOLERANCE

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Aim: To investigate the secretion of the incretin hormone GLP-1 in NIDDM. **Methods:** A 4 hour mixed meal stimulation (2200 kJ) was carried out in 54 NIDDM patients (males/females: 44/10; age: 56±1 yrs; BMI: 30.2±0.7 kg/m^2; HbA1c: 8.4±0.2%; diabetes duration: 4.7±0.8 yrs), and in 33 control subjects with normal glucose tolerance according to WHO criteria and similar medians, means, and ranges for gender, age and BMI (males/females: 27/6); age: 56±1.6 yrs; BMI: 29.6±1.1). Plasma concentrations of glucose, GLP-1, insulin, C-peptide, glucagon, pancreatic polypeptide (PP), gastric inhibitory polypeptide (GIP), and dipeptidylpeptidase IV activity (DPPIV, which inactivates GLP-1) were measured. **Results:** The GLP-1 response, in terms of AUC from 0 to 240 min after start of the meal, was significantly decreased in the patients (2483±145 (patients) as compared to 3145±204 pmolxmin (controls), p=0.008). Also AUC for GIP was decreased (13377±735 (patients) compared to 15991±1267 pmolxmin (controls); p = 0.044, non-parametric test). Multipel regression analysis with total AUC for GLP-1 as dependent parameter and diabetic state, age, BMI, gender, insulin sensitivity and beta-cell function (Homa model), DPPIV activity, HbA1c, fasting plasma glucose, AUC of plasma glucose, and AUC and incremental AUC of insulin, C-peptide, glucagon, PP and GIP showed after backwards elimination a significant negative correlation between AUC GLP-1 and diabetes (p<0.0001), and BMI (p=0.0056), and a significant positive correlation to insulin sensitivity (p=0.0006), and AUC glucagon (p<0.0001). In the model GLP-1 responses were lower in men (p=0.0046). This model explained 43 % of the variability in the GLP-1 response of the subjects. **Conclusion:** We conclude, that the meal-related GLP-1 response in NIDDM patients 1) is decreased, and 2) that it is inversely related to diabetes, and BMI, and positively to insulin sensitivity and glucagon. The decreased GLP-1 response may contribute to the diabetic state.

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MEASUREMENTS OF SATIETY AND FULLNESS FOLLOWING A SUSTACAL CHALLENGE IN TYPE 2 DIABETIC SUBJECTS ADMINISTERED SYNTHETIC EXENDIN-4 (AC2993).

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Exendin-4, a 39 amino acid peptide identified in salivary secretions and the circulation of *Heloderma suspectum*, shares 53% structural identity and several biological actions of glucagon-like peptide-1 (GLP-1), including insulinotropic, glucagonostatic and gastric inhibitory effects. Unlike GLP-1, synthetic exendin-4 (AC2993) has a prolonged duration of action and an ability to inhibit food intake after peripheral injection in rodents, and is being explored as an antidiabetic therapy. To assess a possible effect on satiety in man, 8 non-insulin-using type 2 diabetic patients reported assessments of satiety and fullness on a 100mm visual analog scale at 30 and 60 min intervals for 6 hours following a 7 kcal/kg Sustacal® challenge and subcutaneous injection of placebo or 0.1, 0.2, 0.3 or 0.4 μg/kg doses of AC2993 (n=9, 8, 7, 7, 5) administered in a placebo-balanced dose-rising design with 48 hours between doses. Reports of nausea were also collected. Reported satiety and reported fullness increased with placebo and with all doses of AC2993 following the Sustacal® challenge, reaching maximal sensation at t=60-120 min. Reported satiety returned to pre-challenge, pre-dose values at ~t=6 hours except for the 0.4 μg/kg dose where satiety was still elevated at 8 hours. Reported fullness increased and then returned to baseline 6.5 hours after Sustacal® for placebo treatment, 7.5 hours later for 0.1 and 0.2 μg/kg doses, 8 hours for the 0.3 μg/kg dose, and remained elevated at 8 hours for the 0.4 μg/kg dose. In addition to the general Sustacal®-associated change in satiety and fullness, there was a sustained AC2993-associated dose-dependent increment, especially in the fullness sensation. In contrast to assessments of satiety and fullness, reported nausea was early in onset, peaking within 30 min with the 0.4 μg/kg dose of AC2993 and declining thereafter. In conclusion, these data suggest that AC2993 evokes sustained sensations of satiety and fullness after subcutaneous injection in type 2 diabetic patients, and that these sensations can be dissociated from sensations of nausea.

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SYNERGISTIC INSULINOTROPIC ACTION OF D-GLUCOSE PENTAACETATE AND GLP-1 IN RATS

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Aims: Selected monosaccharide esters were recently proposed as potential new insulinotropic tools in the treatment of type 2 diabetes. The individual and combined effects of one of these esters, α-D-glucose pentaacetate (GPA), and GLP-1 on insulin secretion were now investigated in fed male anaesthetized Wistar rats. **Methods:** GPA (1.7 mmol/l in saline) was first injected over 30 s in an amount of 8.5 nmol per g body wt., and then infused for 12 min at a rate of 1.7 nmol.min^{-1} per g. GLP-1 (2.0 μmol/l in saline containing 1 % [w/v] human serum albumin) was injected over 30 s in an amount of 5 pmol per g 2 min after the start of the GPA infusion. **Results:** The administration of GPA increased within 150 s the plasma insulin concentration by 1.59 ± 0.56 ng/ml (n = 10; P < 0.025), whilst that of saline failed to do so (P > 0.5). The further rise in insulin concentration recorded 2 and 5 min after injection of GLP-1 was also more pronounced in the rats receiving GPA than in its absence, the latter value averaging no more than 63.9 ± 10.8 % (n = 12) of the mean former one. Likewise, at the 2nd and 5th min after injection of GLP-1, the paired increment in the insulinogenic index, i.e. the plasma insulin/glucose ratio, averaged, in the rats not injected with GPA, only 55.8 ± 10.7 % (n = 12) of the mean corresponding value found at the same time in the rats infused with the ester. In these experiments, the plasma insulin concentration reached, 2 to 5 min after GLP-1 injection, mean values of 11.42 ± 2.01 and 5.08 ± 0.48 ng/ml, as distinct (P < 0.001) from a basal value of 2.27 ± 0.24 ng/ml (n = 10-12). **Conclusions:** In normal rats, GPA and GLP-1 act synergistically in increasing plasma insulin concentration. Hence, selected monosaccharide esters could be used to optimize the islet B-cell secretory response to GLP-1 in non-insulin-dependent diabetes.

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PACAP CONTRIBUTES TO INSULIN SECRETION INDUCED BY GASTRIC GLUCOSE GAVAGE IN MICE

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Aim: To study the physiological contribution of pituitary adenylate cyclase-activating polypeptide (PACAP) to the marked insulin secretion induced by oral glucose. **Method:** Either of the PACAP receptor antagonists, PACAP(6-27) or PACAP(6-38) (1.5 nmol/kg) was injected iv at 5 min before a rapid gastric gavage in overnight fasted anesthetized mice (150 mg/mouse). Blood was sampled before and at five time points after the glucose administration for analysis of insulin, glucose and GLP-1. **Results:** Gastric glucose increased plasma insulin from 257±45 to 1060±118 pmol/l and plasma GLP-1 from 19±2 to 42±4 pmol/l at their maximal at 15 min (both p<0.001, n=18). PACAP(6-27) reduced this insulin response by 32% (p=0.018) without altering the concomitant increase in glycemia and potentiating the GLP-1 levels to 62±7 pmol/l (p=0.041). The subsequent glucose elimination was diminished by PACAP(6-27). The other PACAP antagonist, PACAP(6-38), inhibited the insulin response to gastric glucose by the same degree. Combination of the GLP-1 antagonist, exendin$_3$(9-39) (30 nmol/kg) and PACAP(6-27) (1.5 nmol/kg) reduced the insulin response to gastric gavage by 37% (p=0.009). To verify the specificity of PACAP(6-27), the antagonist was found to inhibit the insulin response to iv PACAP27 (1.3 nmol/kg) without affecting that to glucose alone (1 g/kg) or glucose together with carbachol (0.5 μmol/kg), GLP-1 (10 nmol/kg), cholecystokinin8 (15 nmol/kg) or gastrin releasing peptide (2 nmol/kg). **Conclusions:** PACAP receptor antagonism inhibits insulin secretion after gastric glucose through a direct action on insulin secretion and not indirectly through altered glucose uptake; this is accompanied by a compensatory increase in GLP-1 secretion. Hence, the study shows that PACAP contributes to the gut-islet axis acting in concert with GLP-1 to allow a normal incretin response.

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SUSTAINED IMPROVEMENT OF GLUCOSE TOLERANCE BY DPP-IV INHIBITION AFTER CHRONIC TREATMENT WITH NVP-DPP728

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Inhibition of dipeptidylpeptidase IV (DPP-IV) has been suggested as treatment for prandial hyperglycemia in type 2 diabetes because DPP-IV is the key enzyme mediating inactivation of GLP-1 *in vivo*. We and others have demonstrated profound improvements in oral glucose tolerance in a number of animal models during DPP-IV inhibition, that correlate with increased levels of active GLP-1 (7-36 amide) and increased insulin responses. **Aims:** Here we investigated the effects on glucose tolerance of chronic treatment with a selective and potent DPP-IV inhibitor (NVP-DPP728). Further, we studied whether chronic treatment would lead to a diminished beneficial effect of DPP-IV inhibition (tachyphylaxis). **Methods:** Male SD rats, rendered insulin resistant by high fat, were provided with NVP-DPP728 in the drinking water (100μmol/100ml). This dose produces >80% inhibition of plasma DPP-IV during the period of food intake (dark phase). After approximately 6 weeks of access to water with or without NVP-DPP728, the animals were implanted with jugular vein catheters. After recovery, the animals underwent an oral glucose tolerance test (1g/kg) after an overnight fast and with access to pure water only. Animals that were on chronic treatment received 100μmol/kg NVP-DPP728 orally, 30 min. prior to the glucose load. **Results:** Glucose tolerance was improved by 52% (2437 *vs.* 1170, p<0.001, see table below, AUC= area under curve). Acute treatment (only) produced a similar improvement (-42%, 2482 *vs.* 1434, p<0.001). **Conclusions:** We conclude that chronic treatment with a DPP-IV inhibitor does not lead to adaptations resulting in tachyphylaxis. Thus, chronic administration of NVP-DPP728 could be a promising therapeutic approach to improve glycemic control in type 2 diabetic patients.

*AUC glucose (in mg/dl*45 min)*	*Acute vehicle*	*Acute NVP-DPP728*
Chronic water alone	2482±150	1170±108
Chronic NVP-DPP728	2437±150	1434±90

OP 22

New Approaches in Diabetes Management

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INSULINOTROPIC EFFECT OF EXENDIN-4 IN HUMANS

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Exendin-4 (E_4) is a peptide found in the saliva of Gila Monsters. It has 53% homology with GLP-1, the most potent naturally occurring incretin. GLP-1 has a very short biological ½ life (2-4'). We previously reported that during a hyperglycemic clamp at a plasma glucose (GL) level of ~11 mmol/l, the 60-120' insulin (IRI) level in normal (N) volunteers was 234±61 and 150±57 pmol/l in diabetics (DM). When GLP-1 was infused (60-120', 1.5 pmol·kg^{-1}·min^{-1}) the corresponding IRI level was potentiated to 1963±295 and 1050±334. Aims: To examine the insulinotropic effect of E_4 in 7 N and 7 DM volunteers during a hyperglycemic clamp. Materials and Methods: N group age range= 24-56 yrs, mean (X)=44; BMI range=20.2-36.4, X=27.8; M/F=3/4. In the DM group, age range=44-74, X=54; BMI=31-47, X=37, M/F=5/2. Basal GL (N=5.5, DM=10.7 mmol/l) was raised by 5.4 mmol/l in each volunteer for 5 hrs. E_4 was infused from 60-120' (0.15 pmol·kg^{-1}·min^{-1}). Results: Plasma IRI (pmol/l) and glucagon (IRG, pmol/l) levels and GL utilization rates (M, μmol·kg^{-1}·min^{-1}) were: (X±SE)

	N			DM		
	0-60'	60-120'	240-300'	0-60'	60-120'	240-300'
GL	11.1±0.3	10.6±0.0	10.8±0.2	15.9±1.3	16.2±1.3	15.9±1.4
IRI	297±110	1914±300	1993±613	151±21	713±188	681±274
IRG	5.7±0.8	4.4±1.0	3.6±0.9	151±21	13.8±2.5	12.0±1.7
M	12.6±2.2	53.1±4.7	87.1±7.9	3.5±1.7	16.3±9.4	25.8±16.7

All volunteers ate a meal at 330'. Postprandial GL did not rise in any volunteer. Basal NEFA in the N and DM group were 0.57±0.05 and 0.63±0.06 mmol/l, and fell to 0.14±0.03 and 0.42±0.09 at 60'. The 120' level was 0.04±0.01 and 0.14±0.03 remained there for the duration of the clamp. Plasma leptin levels increased in the N group after E_4 infusion but not in the DM group. Conclusions: At 1/10 the dose of GLP-1, E_4 is equally potent with a much longer duration of action and is therefore a potential agent for treating type 2 DM.

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AC2993 (SYNTHETIC EXENDIN-4) LOWERED POSTPRANDIAL PLASMA GLUCOSE CONCENTRATIONS IN PEOPLE WITH TYPE 2 DIABETES.

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Exendin-4, a 39 amino acid polypeptide isolated from the salivary secretions of the Gila Monster (*Heloderma suspectum*), shares 53% homology and certain biologic actions of glucagon-like peptide-1 (GLP-1), including its reported abilities to enhance glucose-stimulated insulin secretion, suppress postprandial glucagon concentrations and regulate gastric emptying. Unlike GLP-1, exendin-4 has been shown to have a prolonged duration of glucose lowering action in vivo and is being explored as an antidiabetic therapy. We evaluated the safety, tolerability, and efficacy of synthetic exendin-4 (AC2993) in 7 male non-insulin using patients with type 2 diabetes who had discontinued other antidiabetic therapy for a minimum of 7 days. A balanced, single-blind, dose-rising, placebo controlled crossover design was employed in which each patient received SC injections of placebo and 0.1, 0.2, and 0.3 μg/kg AC2993 48 hours apart. Five of 7 patients also received a 0.4 μg/kg dose. Plasma glucose, concentrations were assessed fasting and in response to 7 Kcal/kg Sustacal® administered at the time of AC2993/placebo injection. No safety issues were identified from repeated hematology, blood chemistry, urinalysis and ECG measurements. The placebo, 0.1 and 0.2 μg/kg doses were well tolerated. Doses of 0.3 and 0.4 μg/kg elicited a dose-dependent increase in nausea and vomiting occurred at the highest dose. Mean peak plasma AC2993 concentrations rose dose-dependently and were detectable for 15 hours at doses of 0.2 μg/kg and above. For all AC2993 doses, mean plasma glucose concentrations peaked in response to Sustacal at 30-45 minutes and returned to below baseline within 90-120 minutes. For placebo, mean plasma glucose concentrations continued to rise through 90 minutes and did not return to baseline until 6 hours post- Sustacal®. Mean change in plasma glucose (0-300 min) was +75, –5, +3, –5, and –17 mg/dl for PBO, 0.1, 0.2, 0.3, and 0.4 μg/kg respectively (P <0.02 vs. PBO). The lowest plasma glucose recorded was 83 mg/dl. In conclusion, SC injection of AC2993 to patients with type 2 diabetes, identified no safety issues, was well-tolerated at doses 0.2 μg/kg, led to detectable AC2993 plasma immunoreactivity for up to 15 hrs and decreased plasma glucose in all tested doses without inducing hypoglycemia. Thus, these observations support the continued exploration of AC2993 for the treatment of type 2 diabetes.

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THE EFFECTS OF A SPECIFIC GLUCAGON ANTAGONIST ON GLUCAGON STIMULATED GLUCOSE PRODUCTION

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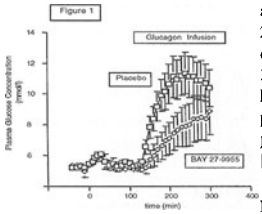
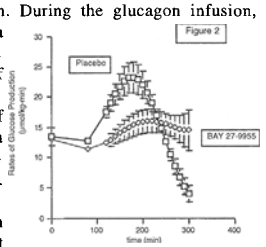
Aim: To examine the efficacy of a novel glucagon antagonist in humans. Methods: We studied 16 young, healthy men before and after a single dose (200mg p.o.) of a selective glucagon antagonist (BAY 27-9955). During both studies, basal glucose production (GP) was measured after a 12 hr fast with a primed-continuous infusion (0.05mg/kg·min) of [6,6-^{2}H]glucose. At t=120 minutes, a 3 hr infusion of somatostatin (0.1µg/kg·min) was initiated in order to suppress endogenous glucagon and insulin release. Insulin was infused at 24pmol/m^2·min to replace basal insulin concentrations and glucagon was infused at a rate of 3ng/kg·min in order to induce selective hyperglucagonemia. Results: During this infusion plasma [glucagon] increased from 50 pg/ml to 100 pg/ml (p<0.001). During the basal state, plasma [glucose] remained constant at 5mmol/l and GP was 13µmol/kg·min. During the glucagon infusion, plasma [glucose] increased immediately to a peak of ~12 mmol/l (Fig 1) and GP increased by a factor of almost 2 from 12µmol/kg·min to 23µmol/kg·min (p<0.001) (Fig 2). In the studies where 200mg of BAY 27-9955 was given, the increase in plasma [glucose] was blunted (peak ~8mmol/l) (p<0.001) and GP remained unchanged as shown in Fig 2. There were no side effects of this drug at this dose.





Conclusion: During a physiologic increase in plasma [glucagon], BAY 27-9955 almost completely blocked the glucagon induced increase in hepatic GP. Given the well established relationship between fasting plasma [glucose] and rates of GP in patients with diabetes, this novel class of glucagon receptor antagonists may prove very useful in the treatment of patients with type 2 diabetes.

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APPLICATION REGIMES OF DIPEPTIDYL PEPTIDASE IV INHIBITORS TO IMPROVE GLUCOSE TOLERANCE IN RATS

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Aims: Isoleucine thiazolidide (IT) protects insulinotropic potency of intestinal incretins from dipeptidyl peptidase IV (DPIV) inactivation after meals and improves glucose tolerance (Gt). DPIV inhibitors are considered as a new tool to treat type 2 diabetes. We clarified dose effect characteristics of the IT drug form P31/98. We also applied the chemically more stable drug P32/98 with comparable plasma DPIV inhibiting capacity to elucidate optimal timing of drug application before oral glucose loads (OGTT). - **Material and Methods:** Male Wistar rats (≥ 350 g body weight) were instrumented with chronic catheters (*A.carotis*). P31/98 (1.25, 2.5, 5, 7.5 ... 50, 100 µmol/300 g b.w.) dissolved in 40 % glucose solution was orally given (S I). In addition 2.5 µmol P32/98 per 300 g b.w. were given orally at 0, 5, 10, 20, 40 or 60 min before OGTT (S II). Control animals (C) received the OGTT. Plasma DPIV-activity, insulin, C-peptide and glucose were measured during 2 h OGTT (2.0 g/kg b.w.; n=5 each group). - **Results:** In S I plasma DPIV-acitivity was dose-dependently diminished at P31/98 doses of 10 to 100 µmol/300 g b.w. (C: 14±3, 10 µmol/300 g: 55±3, 100 µmol/300 g: 88±2 %; means±SEM; p<0.05). 5.0 µmol/300 g b.w. induce an early and higher insulin peak than C (C 16±2, 13±4; 10±0 min, 32±10 ng/l; S), lowest glucose area under the curve (AUC 0-60 min; C 161±26; 110±12 mmol·min/l, S) and reduce C-peptide AUC slightly (C 228±46; 168±36 nmol·min/l; NS). To improve Gt a dose dependency was proven for low doses (cubic regression analysis: r=0.676; S). In S II P32/98 application before OGTT induced improvement in glucose AUC (0: 135±16, 5: 108±13, 10:90±21, 20: 77±16, 40: 71±20, 60 min: 107±29 mmol·min/l; S for 10 to 40 min). - **Conclusions:** 1. DPIV inhibitors improve Gt by early and increased insulin secretion. This seems to save insulin. 2. 5.0 µmol P31/98 or 2.5 µmol P32/98 per 300 g b.w. are most effective in improving Gt. 3. DPIV inhibitors given 20 to 40 min before OGTT further improve Gt.

OP 23

Neuropathy-Mechanisms and Pathogenesis

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A NEUROPATHOLOGICAL BASIS FOR PAIN IN DIABETIC AMYOTROPHY.

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Aims: Pain is a major feature of diabetic amyotrophy and its duration suggests significant structural as opposed to metabolic abnormalities. **Materials and Methods:** We have studied 10 Type II diabetic patients (Age- 65.4 ± 7.2 yr., duration of diabetes- 10.2 ± 7.6 yr., duration of amyotrophy-4.2 ± 1.8 months, HbA1c-7.7 ± 1.3%) with amyotrophy (DA), 14 diabetic patients (DN) with an equivalent degree of neuropathy without amyotrophy, and 14 control subjects (C). **Results:** Diabetic patients with amyotrophy had marked pelvifemoral weakness (MRC-15.9 ± 3.5/30) and pain (Visual Analogue Score of 7.8 ± 1.6/10). The intermediate cutaneous nerve of the thigh (ICNT) and sural nerve were biopsied in DA and the sural nerve alone in DN and C. Myelinated fibre density (no.mm^{-2}) was significantly reduced in the ICNT (3104 ± 181, P<0.0004) and sural nerve (2756 ± 454, P<0.0009) of DA v DN (4042 ± 539) and C (6049 ± 342). Myelinated fibre area (µm^2) did not differ significantly between ICNT (27.7 ± 2.4) and sural (28.9 ± 2.2) of DA v DN (31.8 ± 1.9) or C (29.1 ± 1.6). However, myelinated fibre axonal area (µm^2) was significantly reduced in the ICNT (5.9 ±0.4, P<0.0001) and sural (7.1 ± 0.3, P<0.0008) of DA v DN (10.8 ± 0.8) and C (10.5 ± 0.8). Unmyelinated fibre degeneration (%unassociated Schwann cell profiles) was increased in ICNT of DA (47.8 ± 5.6) (p<0.002) v the sural of C (20.6 ± 1.9) but was not different from the sural of DN (57.9 ± 4.5). Unmyelinated axon density was significantly increased in the ICNT of DA (101020 ± 9513) v C (64116 ± 4889) (p<0.005) and axon diameter was reduced in ICNT of DA (0.58 ± 0.04) v C (0.83 ± 0.02) (p<0.0005). **Conclusions:** Diabetic amyotrophy is characterised by a distal loss of myelinated fibres with axonal atrophy and unmyelinated fibre regeneration. Such significant structural as opposed to functional changes may account for the severe and protracted pain in this condition.

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REGIONAL SYMPATHETIC DENERVATION IN PATIENTS WITH PAINFUL DIABETIC NEUROPATHY

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Aims: Pain in peripheral diabetic neuropathy has been thought to be related to abnormal sympathetic nerve activity in the affected limbs, and drugs that interact with sympathetic neuronal function like desipramide and clonidine have documented effect in pain treatment. To investigate the function of the peripheral sympathetic nervous system in more detail in vivo, we combined neurochemical measurements with functional positron emission tomography (PET).

Methods: Nine patients with diabetes mellitus (M:F = 4:5, age 61 ± 7 years) and painful peripheral neuropathy of the feet, but without generalized autonomic neuropathy, underwent i.v. infusion of titrated norepinephrine (NE) and sampling of arterial and venous blood in both feet and in one arm, before and during ganglion blockade with trimethaphan. PET scanning after i.v. ^{13}N-ammonia was used to image local perfusion and i.v. 6-[^{18}F]fluorodopamine to image sympathetic nerve innervation. The results were compared with those in the limbs of normal volunteers (n=12), and in the unaffected leg (n=15) or unaffected arm (n=16) of patients with unilateral complex regional pain syndrome (CRPS).

Results: Regional NE spillover in the feet was significantly less in the group with painful diabetic neuropathy than in the control groups and significantly less in the affected feet than in the unaffected arms of the control patients of subjects. After trimethaphan, foot NE spillover decreased more in the control groups than in the patients with diabetic neuropathy. Reported pain scores in patients with diabetes did not change during ganglion blockade. PET scanning revealed decreased flow-corrected 6-[^{18}F]fluorodopamine-derived radioactivity in patients with diabetic neuropathy, compared to values in normal volunteers

Conclusions: These results provide neurochemical evidence, supported by sympatho-imaging findings, for selective regional (partial/selective) denervation of the sympathetic nervous system in the legs of patients with painful diabetic neuropathy. Pain in these patients appears not to be sympathetically-mediated.

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EVALUATION OF AN ARI ON DIABETIC NERVE FUNCTION, METABOLISM AND ANTIOXIDANT STATUS: AN INTERVENTION STUDY

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Aims. ARIs prevent peripheral nerve dysfunction in experimental diabetes. However, some intervention studies (both experimental and clinical) appeared disappointing which could reflect either their inadequate design and insufficient correction of the sorbitol pathway activity, or the ineffectiveness of ARIs in *reversal* of functional and metabolic deficits of diabetic neuropathy in general. We evaluated if diabetes-induced changes in nerve function, metabolism and antioxidant status can be corrected by the dose of ARI (sorbinil, 60 mg/kg/d in the diet) resulting in complete inhibition of increased nerve sorbitol pathway activity. Materials and Methods: The groups included control (C) and STZ-diabetic (D) rats treated with or without ARI for 2 wks after 4 wks of untreated diabetes. Sciatic endoneurial nutritive blood flow (NBF) was measured by hydrogen clearance. Sorbitol pathway and other metabolites, phospho-creatine (PCr), creatine (Cr) and ATP were assayed by enzymatic methods, GSH and ascorbate (AA) fluorometrically. Results: NBF($ml*min^{-1}*100g^{-1}$) and motor and sensory nerve conduction velocities (MNCV and SNCV, m/s) were decreased in D (9.3±0.8, 44.7±1.2 and 32.3±0.4 vs. 17.6±1.3, 57.0±1.7 and 38.4±1.0 in C, $p< 0.01$), and this decrease was corrected in D+ARI (14.0±0.6, 54.3±1.3 and 36.6±1.0, $p<0.05$ vs.D). Free mitochondrial and cytosolic $NAD^+/NADH$ ratios (assessed from β-hydroxybutyrate and lactate dehydrogenase systems), PCr levels and PCr/Cr ratio were decreased in D vs.C, and both $NAD^+/NADH$ ratios and PCr levels were ameliorated but not normalized by ARI while PCr/Cr was completely restored. GSH and AA levels were decreased in D, and only GSH but not AA levels were normalized by ARI. Conclusions: ARI treatment is an effective approach for restoration of NBF, MNCV and SNCV, and at least partial correction of nerve metabolic and antioxidant deficits in diabetes. It remains to be established if residual metabolic and antioxidant imbalances 1) are of any importance for development of diabetic neuropathy and 2) can be corrected by longer ARI treatment or are due to AR-independent mechanisms.

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POLYOL LEVELS AND MORPHOLOGY OF SURAL NERVE IN RELATION TO NERVE FUNCTION AND NEUROPATHY

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Aims: Relate sorbitol and *myo*-inositol levels and morphology of sural nerve to nerve function and clinical neuropathy in men with diabetic, impaired (IGT), and normal glucose tolerance.

Materials and Methods: After neurophysiological examination, sural nerve biopsy was performed in 10 men with Type 1 diabetes, 10 with IGT, and 10 with normal glucose tolerance.

Results: Sural nerve conduction velocities (median [IQ range]) (41.0 [6.0] m/s vs 47.0 [3.0] m/s; P = 0.014) and amplitudes (SNAP) (3.7 [3.5] μV vs 11.3 [10.6]) μV; P = 0.04) were lower in diabetic than in IGT subjects. Sorbitol levels were higher in diabetic subjects (643 [412] nmol/mg prot.) compared with IGT (286 [83] nmol/mg prot. P = 0.00032) and normal subjects (296 [250] nmol/mg prot.; P = 0.0191). Nevertheless, there was a negative correlation (r = -0.69; P =0.0376) between SNAP and sorbitol levels in IGT. Nerve morphology did not differ between the three groups. In diabetic and IGT subjects, however, myelinated fiber density (MNFD) was (P = 0.0021) lower in 9 subjects with clinical neuropathy (4076 [1091] nr fibers/mm^2) than in 10 without (5219 [668] nr fibers/mm^2). *Myo*-inositol levels were lower (p = 0.0283) in diabetic patients with clinical neuropathy (25049 [3985] nmol/mg prot) than in those without (32253 [7503] nmol/mg prot).

Conclusions: High sorbitol levels were associated with disturbed nerve function in IGT, low *myo*-inositol levels with clinical neuropathy in diabetes, and reduced MNFD correlated with clinical neuropathy but not with glucose tolerance status.

OP 24

Epidemiology of Type 2 Diabetes I

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ISOLATED POST-CHALLENGE HYPERGLYCAEMIA INCREASES THE RISK OF MORTALITY

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Aims: The aim of this study was to examine the possible link between isolated post-challenge hyperglycaemia (IPH) (plasma glucose ≥11.1 mmol/l, 2h after a 75g oral glucose load, and fasting plasma glucose <7.0 mmol/l) and mortality. **Materials and Methods:** The data from three population based longitudinal studies (in Mauritius, Fiji and Nauru) were pooled, and mortality rates were determined in 9179 people, who were followed for between 5 and 12 years. Vital status was available for 99% of the cohort. **Results:** At baseline, there were 595 people with previously diagnosed diabetes, and 799 with newly diagnosed diabetes (fasting plasma glucose ≥7.0 mmol/l, or 2h plasma glucose ≥11.1 mmol/l), of whom 243 (31%) had IPH. In comparison to people without diabetes, people with IPH had an increased risk of all cause mortality (Cox proportional hazards ratio [95%CI] adjusted for age and other risk factors: 2.7 [1.8-3.9] - men; 2.0 [1.3-3.3] - women), and of cardiovascular mortality (2.3 [1.2-4.2] - men; 2.6 [1.3-5.1] - women). These risks were at least as strong as for those people who were diabetic on both the fasting and 2h values (n=404), for all cause mortality (1.9 [1.4-2.6] - men; 1.5 [1.0-2.3] - women), as well as for cardiovascular mortality (1.7 [1.0-2.8] - men; 1.7 [0.9-3.2] - women]). In addition, men with IPH had a high risk of cancer death (8.0 [3.6-17.9]). The median fasting glucose in people with IPH was 6.0 mmol/l (5.7 mmol/l in those who died). **Conclusions:** People whose only glucose abnormality is in the post-challenge state form a significant proportion of the diabetic population, and have an increased mortality risk. This should be considered in the design of screening programmes that use only the fasting glucose.

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INFLUENCE OF GENDER ON CARDIOVASCULAR RISK FACTORS AND INSULIN RESISTANCE IN IMPAIRED FASTING GLUCOSE.

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Aims: To compare the levels of cardiovascular risk factors and insulin resistance assessed by HOMA (Homeostasis Model Assessment) in men and women affected by impaired fasting glucose (IFG, 6.1-6.9 mmol/L). **Materials and methods:** In a random sample of 3508 subjects, aged 35-64 yrs, participating in the French MONICA study between 1995 and 1997, IFG affects 88 women and 181 men. According to standardized MONICA protocol, these subjects underwent clinical and blood pressure (BP) measures, then centralised biological determinations were performed on fasting blood sample. **Results :** In univariate analysis, physical activity level ($p<0.01$), alcohol consumption (35.2 vs 9.3 g/day, $p<0.001$), waist circumference (99.8 vs 93.2 cm, $p<0.001$), diastolic BP (87 vs 83 mmHg, $p<0.01$) and triglycerides (1.61 vs 1.35 mmol/L, $p<0.05$) are higher in men than in women, whereas age (51 vs 55 yrs, $p<0.001$), BMI (28.2 vs 29.5 kg/m^2, $p<0.05$) apoA1 (160 vs 168 mg/dL $p<0.05$) and HDLc (1.29 vs 1.50 mmol/L, $p<0.001$) are lower in men than in women. Systolic BP, total cholesterol, LDLc, apoB, fasting insulinemia and HOMA values are similar in both sexes. After multivariate adjustment including age, alcohol consumption, physical activity level, BMI and waist circumference, a significant difference remains only for HDLc ($p<0.01$). The number of actual and ex-smokers is higher in men than in women (23.2% and 50.3% vs 14.8% and 15.9%, $p<0.001$). The prevalences of hypertension (antihypertensive treatment or sBP≥140 or dBP≥90 mmHg) and dyslipidemia (hypolipemic treatment or LDLc≥4.1 or triglycerides≥2.3 mmol/L) reaches respectively 58% and 32% in men, 56% and 38% in women (ns). **Conclusions:** IFG is associated with high cardiovascular risk factors level in both sexes. No difference in cardiovascular risk factors and insulin resistance profiles appears between men and women except for HDLc and tobacco consumption.

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EVALUATION OF THE 1997 A.D.A AND 1998 W.H.O. DIAGNOSTIC CRITERIA FOR DIABETES IN A CHINESE POPULATION

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Aims: We evaluated, in this study, the relative merit of the new diagnostic criteria for diabetes proposed by the American Diabetes Association (ADA 1997) and the World Health Organisation (WHO 1998). Although both organisations agreed that the diagnostic fasting plasma glucose (FPG) for diabetes should be lowered from 7.8 mmol/L to 7.0 mmol/L, the ADA 1997 diagnostic criteria are based on FPG only, whereas the WHO criteria retain the use of the standard oral glucose tolerance test (OGTT). **Materials and Methods:** We analysed the data collected from the Hong Kong cardiovascular Risk Factor Prevalence Study using both sets of diagnostic criteria and those proposed by the WHO in 1985. This population-based study was conducted from 1995 to 1996 and included a 75-g OGTT performed in 2752 Chinese subjects aged 25 to 74 years. **Results:** Based on the 3 sets of criteria, the prevalence of diabetes (known and unknown) was 7.3% (ADA 1997), 9.7% (WHO 1998) and 9.3% (WHO 1985) respectively. Subjects diagnosed as diabetic by the WHO 1998 criteria, but not when the ADA 1997 criteria were used, had adverse cardiovascular risk profiles which were comparable to those classified as diabetic by both. Of those subjects classified as normal by the ADA 1997 criteria, 15% had impaired glucose tolerance and 2% had diabetes using WHO 1998 criteria. These subjects with abnormal 2h-PG only had unfavorable cardiovascular risk profiles compared to those classified as normal by both, with those with diabetes having cardiovascular risk profiles comparable to subjects with impaired fasting glucose (IFG), the new category of glucose intolerance introduced by the ADA. They would be missed if FPG alone was tested. **Conclusion:** These data are in support of the WHO recommendation that the OGTT be retained to maximally identify at risk individuals.

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DIFFERENCES BETWEEN IMPAIRED FASTING GLUCOSE AND IMPAIRED GLUCOSE TOLERANCE: ASSOCIATED RISK FACTORS IN THE POPULATION

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Aims: To compare the new "impaired fasting glucose" (IFG) diagnostic category (ADA, 1997) with the classical "impaired glucose tolerance"(IGT) category, by means of a population study, with reference to the associated cardiovascular risk factors. **Methods:** These categories were compared by a random stratified sampling in a representative council of our area. 691subjects had an standard OGTT performed in the fasting state (excluding those who had been previously diagnosed as having diabetes mellitus or had fasting glucose > 7.8 mM/L). Height, weight, waist and hip perimeters and blood pressure were measured; fasting lipid profile (triglycerides, total and HDL cholesterol) was determined. Medication and tobacco use was also reported. **Results:** 118 subjects (17.1%) had IGT and 61 (8.8%) had IFG. Only 22 subjects (3.2%) had both IGT and IFG. Of the subjects with IGT, 91 (77.1%) were normal, 22 (18.6%) had IFG and 5 (4.2%) had DM according to the new ADA criteria. Of the subjects with IFG, 28 (45.9%) were normal, 22 (36.1%) had IGT and 11 (18.0%) had DM according to the classical criteria. Cohen's kappa for concordance was 0.531. A multiple logistic regression study showed that the risk factors associated with IFG were: body mass index (p=0.021), diastolic blood pressure (p=0.44) and (marginally) systolic blood pressure (p=0.051) without association with age, sex, tobbaco use, waist/hip ratio or lipid profile. With IGT the associations were: age (p=0.001) and (marginally) body mass index (p=0.060) and plasma triglycerides (p=0.097) without association with sex, tobacco use, blood pressure, waist/hip ratio or cholesterol. **Conclusions**: We conclude that the overlapping of IFG and IGT is poor; besides, the cardiovascular risk factors associated with these categories appear to be different in our population. Therefore, the substitution of IFG for IHC would be inadequate.

OP 25

Insulin Sensitivity and Triglycerides

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EFFECT OF TROGLITAZONE ON GLUCOSE METABOLISM AND INSULIN SENSITIVITY IN RELATIVES OF TYPE 2 DIABETIC PATIENTS.

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Aims: We investigated the effect of troglitazone (TGZ) on glucose metabolism, insulin sensitivity and glycogen synthase activity in first degree relatives of type 2 diabetic patients. **Methods:** The relatives were randomized in a double blind manner and treated for 12 weeks with either TGZ 200 mg or placebo. Before and after treatment an oral glucose tolerance test (OGTT) and an euglycaemic hyperinsulinaemic clamp (40mU/m^2/min) were performed. The latter including tritiated glucose (HOT-GINF), indirect calorimetry and muscle biopsies for enzyme analyses. **Materials:** Twelve relatives (f/m:6/6) receiving TGZ and 11 (3/8) relatives receiving placebo (age: 30,8± 2,0 vs 30,6±1,7 yr, BMI. 29,6±0,8 vs 30,6±1,4 kg/m^2) were compared. **Results:** Data from OGTT showed that no changes occurred in weighted mean p-glucose in the TGZ group compared to the placebo group during treatment (7,8±0,5 to 7,9±0,5 vs 7,9±0,3 to 8,1±0,5 mmol/l, NS). Also IGT status (3 in each group) remained unchanged. However, weighted mean p-insulin decreased in the TGZ group compared to placebo (394,8±84,6 to 348,6±91,4 vs 381,8±59,7 to 493,7±88,9, p<0,05). Insulin sensitivity as measured by the glucose infusion rate during insulin stimulation increased in the TGZ group (164,5±24,3 to 218,2±32,1 vs 149,5±23,3 to 154,6±23,5 mg/min/m^2, p<0,01) No significant differences in insulin stimulated glucose and lipid oxidation calculated from indirect calorimetry were found (glucose ox from 125,2±11,6 to 136,8±12,4 vs 112,8±5,7 to 107,8±8,2mg/min/m^2, NS), (lipid ox from 16,7±5,4 to 13,0±5,9 vs 25,0±2,8 to 27,7±3,6 mg/min/m^2, NS). Glucose storage (Rd - glycolytic flux (calculated from tritiated water)) on the other hand, increased significantly in the TGZ group (98,2±18,1 to 140,0±23,9 vs 83,5±16,3 to 89,4±15,9mg/min/m^2, p<0,05) This increase however, was not reflected in a simultaneous increase in glycogen synthase activity ($FV_{0,1\ G6P}$. 0,43±0,03 to 0,43±0,04 vs 0,42±0,04 to 0,47±0,03 %, NS) **Conclusion:** Treatment with troglitazone significantly increases insulin sensitivity in relatives of patients with Type 2 diabetes and this effect seems mainly to be located to the glucose storage pathway of glucose metabolism. However, this seems not mediated via an increased insulin activation of glycogen synthase.

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PPARγ2 AMINO ACID POLYMORPHISM PRO12ALA ALTERS THE CORRELATION BETWEEN INTRAMYOCELLULAR LIPID CONTENT AND INSULIN SENSITIVITY IN OFFSPRING OF TYPE 2 DIABETES PATIENTS

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The peroxisome proliferator activated receptors and its mutations could play a role in the pathogenesis of obesity and type 2 diabetes mellitus. Lately a mutation in the coding sequence of isoform PPARγ2 was described, with a substitution of Prolin to Alanin at codon 12 (Pro12Ala). Recently, we and others found a close and negative correlation between insulin sensitivity (IS) and intramyocellular lipid content (IMCL) in lean offspring of type 2 diabetes patients (OFF-DM). **AIM and METHODS:** To see whether this Pro12Ala mutation affects the association of increased IMCL and insulin resistance, we selected a group of 12 lean subjects, heterozygous for this mutation (Pro12Ala), from more than 300 OFF-DM who had undergone extensive metabolic phenotyping. To minimize confounders, the control group without this mutation (C) was matched according to sex, BMI, fat mass and body fat distribution (WHR). IS was assessed by a standard hyperinsulinemic glucose clamp, with M/I as an index of insulin sensitivity; in addition IMCL and total muscular lipid (TML) were quantified by proton magnetic resonance spectroscopy in two muscles of the calf, the tibialis anterior(TA) and the soleus muscle (SOL). **RESULTS:** Due to matching there were no significant differences between both groups. IMCL and TML in both muscles were similar or tended to be even higher in Pro12Ala. However, despite this, IS was markedly higher in Pro12Ala, also fasting Insulin was significantly lower (*p<0.05).

	IMCL-TA	TML-TA	IMCL-SOL	TML-SOL	Insulin µU/ml	M/I mg/kg*min/µU/ml
Pro12Ala	3,4	12,0	9,3	23,9	5,5*	14,84*
C	2,8	9,5	8,7	20,2	8,5	8,63

CONCLUSION: In this well matched group of lean subjects, Pro12Ala had similar or even slightly higher IMCL and TML in both muscles; however, inspite of this, insulin sensitivity was markedly higher (+72%). One could therefore speculate that the Pro12Ala mutation has some protective role and reduces the negative impact of IMCL depots on insulin sensitivity.

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OSCILLATING CONCENTRATIONS OF FREE FATTY ACIDS INDEPENDENT OF INSULIN AND GLUCOSE OSCILLATIONS

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Aim. Glucose and insulin concentrations exhibit significant oscillations at a periodicity of 5 to 15 minutes, due to pulsatile insulin secretion, and oscillatory hepatic glucose output, correlating to pulsatile release of glucagon and somatostatin. Recent data support an important role of free fatty acids (FFA) on insulin release. Conversely, adipose tissue is highly insulin sensitive, suggesting possible oscillatory lipolysis due to pulsatile insulin release. The aim of the present study was to examine 1) if oscillations in FFA occur with enhanced lipolysis (fasting), and at basal conditions, and 2) if such oscillations may be entrained by glucose induced pulsatile insulin secretion. **Materials and methods.** In protocol 1 7 healthy subjects were studied at basal (10 h fast) and long term fasting (60 h fast) conditions. In protocol 2 the relation of glucose and insulin oscillations to FFA was examined in 6 subjects, where glucose (6 mg/kg/min) was infused over 1 min every 7 or every 12 min for 60 mun after which frequency was abruptly altered to opposite frequency for additional 60 min. In both protocols minutely sampled blood was analyzed in triplicate for insulin (ELISA, CV ~3 %), glucose (glucose oxidation, CV~5%) and FFA (enzymatic colorimetric method, CV ~2 %) concentrations. Data was analyzed for significant oscillations applying autocorrelation (AC) and spectral analysis (SA) to first differenced concentration time series. **Results.** At long term fasting significant ($p<0.05$) oscillations in FFA were detected in 6/7 subjects by SA (periodicity 3.7 ±0.2 min), and in 4/7 subjects by AC (periodicity 3.8 ±0.3 min). At basal condition similar periodicities (3.8 ±0.2 min) were detected by SA in 5/7, but only in 1/7 by AC (periodicity 3.5 min). The detected periodicities of FFA oscillations were significantly faster than insulin oscillations (7.1 ±0.3 min by AC) Infusion of glucose at 7 or 12 min interval resulted in significant (<0.05 by AC) oscillations in glucose (~5 %) and insulin (~100 %) at identical periodicity in all studies. In contrast, no FFA oscillations occurred at expected periodicities (7 or 12 min) by SA or AC, whereas a dominant peak was observed in 7/12 data series at a mean periodicity of 4.0 ±0.2 min. The data demonstrate occurrence of significant oscillations in FFA at a periodicity of ~4 min at long term fasting, and at basal conditions. These oscillations are different in frequency from insulin oscillations, and are not entrainable by short term glucose induction of pulsatile insulin release. An insulin and glucose independent mechanism, likely sympathetic, seems to control these oscillations

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INCREASED CONTRIBUTION OF LIPOGENESIS AND DECREASED CONTRIBUTION OF NEFA REESTERIFICATION TO TRIGLYCERIDES SECRETION IN INSULIN-RESISTANCE.

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Aims : Abnormalities of NEFA and triglycerides (TG) metabolism are observed in insulin-resistance and diabetes. Thus it is important to measure simultaneously the main pathways of NEFA and TG metabolism in human subjects. **Protocol** : 5 normal subjects (BMI 22.7±0.9 kg/m2) and 5 insulin-resistant (IR) patients (BMI 33.4±3.4 kg/m2) drank deuterated water (for measurement of hepatic de novo lipogenesis) in the evening and were infused (four hours) the following morning in the post-absorptive state with [1-13C]palmitate. We determined : 1) NEFA turnover rate (Rt), 2) NEFA oxidation rate, 3) total lipid oxidation (indirect calorimetry), 4) intrahepatic reesterification (from the kinetic of 13C-palmitate incorporation into TG during [1-13C] palmitate infusion), 5) TG turnover rate (from the kinetic of 13C-palmitate disappearance in TG after the end of [1-13C] palmitate infusion). **Results** : NEFA concentration was higher in IR (0.67±0.12 vs 0.28±0.06 mmoles in control subjects,$p<0.01$). NEFA Rt was similar in the two groups (6.04±1.17 and 5.23±0.8 μmoles/kg/min in normal and IR). Using the acetate correction factor of Sidossis NEFA oxidation rate was similar in the two groups (2.8±0.7 and 2.3±0.5 μmoles/kg/min in normal and IR subjects), accounting for 45 % of NEFA Rt and near all total lipid oxidation (3.02±0.54 and 2.47±0.58 μmoles/kg/min for normal and IR subjects). Total NEFA reesterification was 3.28±0.6 (normal subjects) and 2.95±0.59 μmoles/kg/min (IR). TG Rt was similar in IR and control subjects (0.11 μmoles/kg/min). The percent contribution of hepatic lipogenesis to TG Rt was higher in IR (10% vs 4%, $p<0.01$) whereas that of hepatic NEFA reesterification was reduced (25 vs 49 % in normal subjects, $p<0.01$). **Conclusions** : 1) Despite similar TG, Rt IR have an increased contribution of hepatic lipogenesis and a decreased contribution of NEFA reesterification to TG secretion. 2) Despite elevated plasma NEFA in IR, NEFA Rt and oxidation were comparable in the two groups. 4) FFA oxidation account for near the totality of lipid oxidation in the two groups.

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The Exocytotic Machinery of the Islet Cell

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PHOSPHATIDYLINOSITOL-4-PHOSPHATE STIMULATES EXOCYTOSIS IN PANCREATIC B-CELLS BY ACTIVATION OF PROTEIN KINASE C

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Granule-associated phosphatidylinositol-4-kinase activity is required for stimulated secretion and might be an important basis for ATP use in the exocytotic pathway. Here we have explored the effects of phosphatidylinositol-4-phosphate (PtdIns-4P) on Ca^{2+}-evoked exocytosis in mouse pancreatic B-cells using the standard whole-cell configuration of the patch-clamp technique and capacitance measurements of exocytosis. The rate of increase in cell capacitance, elicited by infusion with a Ca^{2+}-EGTA buffer with a free Ca^{2+} concentration of 0.22 μM, increased 2-fold by inclusion of 1 μM PtdIns-4P in the pipette solution. The effect was concentration dependent (EC_{50}=8 nM) and mimicked by PtdIns-3P, PtdIns-4,5P_2, PtdIns-3,4P_2, PtdIns-3,4,5P_3, but not by phosphatidylcholine, inositol 1,4,5-trisphosphate or phosphatidylinositol. The effect of PtdIns-4P on exocytosis was dependent on protein kinase C (PKC) and was blocked by staurosporine and calphostin C. In accordance with these results, PtdIns-4P stimulated PKC activity 2-fold in a 100000g soluble fraction of mouse islets. PtdIns-4P did not affect phospholipase A_2 or D in mouse islet homogenate. These results suggest an important role for phosphastidylinositol-4-kinase in Ca^{2+}-activated secretion. Indeed, exocytosis stimulated by a maximal Ca^{2+} concentration (2 μM) was reduced by 380% by pre-treatment with the phosphatidylinositol-4-kinase inhibitor phenylarsine oxide (20 μM). This block could be reversed by addition of 2,3-dimercaptopropanol (0.1 mM) or PtdIns-4P (1 μM). These data suggest that PtdIns-4P-mediated stimulation of PKC is important for regulated fusion of secretory granules with the plasma membrane. Finally, our data implies that lipid kinase-mediated phosphorylation might represent an important step in ATP-dependent priming of secretory granules in insulin exocytosis.

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Exocytosis in B-cells studied in intact mouse pancreatic islets by a combination of capacitance measurements and electron microscopy.

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We have combined electron microscopy (TEM) and measurements of cell capacitance (to monitor exocytosis) on intact pancreatic islets to determine the correlation between the functional responses and the ultrastructure of the B-cell. A train of nine depolarising pulses (from -70 to 0 mV, duration 500 ms) increased the cell capacitance 660±340 fF (n=4). The total increase in cell capacitance evoked by 10 trains separated by 1 min was 900 fF. This corresponds to the release of 450 secretory granules or 4.1% of the total granule number assuming that there are 11 000 granules per B-cell. The increase in cell capacitance elicited by the first 500 ms depolarisation of each train was 106±48 fF in the presence of 3 mM glucose alone. Interestingly, this is similar to that which can be released from an isolated B-cell in the presence of forskolin but 3-fold larger than that released in the absence of the adenylate cyclase activator. The total number of granules released over 10 min determined by measurements of cell capacitance is comparable to the decrease in the number of docked granules when the cells were exposed to 150 mM K^+ which fell from 9.8±1.0% to 4.9±0.9% (n=14-17) of the total granule number. In conclusion, there is a fair agreement between the ultrastructural (EM) and functional (electrophysiological) measurements. Our data further indicate that the readily releasable pool is a subset of the docked granules and that mobilisation (i.e. the refilling of the readily releasable pool) of the functionally distal part of the reserve pool reflects the chemical modification of the granules rather than their intracellular translocation. Finally, the amplitude of the exocytotic responses observed in the intact islets suggest that paracrine mechanisms are operational already under basal conditions.

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ALTERED β-CELL COUPLING RESULTS IN ABNORMAL INSULIN SECRETION AND GLUCOSE TOLERANCE IN VIVO

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Aim: we have studied whether the intercellular exchange of cytoplasmic ions and molecules that takes place between β-cells coupled by connexin channels is critical for the control of insulin secretion in vivo. **Materials and methods:** to this end, we have generated two lines of transgenic mice that express connexin Cx32, a gap junction protein which is not expressed within native islets, under control of the rat insulin promoter II. Heterozygous and homozygous animals were studied for connexin expression (as evaluated by PCR, immunolabeling and tracer microinjection), insulin secretion (as evaluated by in situ perfusions of pancreas) and glucose tolerance. **Results :** when compared to littermates that did not express Cx32 within islets, transgenic mice 1) expressed abundant levels of this protein in β-cells, 2) featured a largely increased ($p< 0.001$) exchange of membrane-impermeant molecules and cytoplasmic ions between β-cells, 3) had elevated basal insulin secretion ($p< 0.01$), 4) had a much decreased insulin secretion in response to 4.2-16.7 mM glucose ($p< 0.001$), 5) were slightly hypoglycemic under basal conditions ($p< 0.02$), 6) remained hyperglycemic much longer than controls ($p< 0.001$) after an in vivo challenge with glucose (0.1 mg /Kg). These changes were observed in two independent lines of transgenic mice and were more pronounced in homozygous than heterozygous animals. **Conclusions :** the data show that abnormal levels of the communications that occur through connexin channels linking β-cells, cause major defects in insulin secretion that are sufficient to alter the in vivo control of glycemia.

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G_{α} PROTEINS COUPLE SOMATOSTATIN RECEPTORS TO K^+-CHANNEL ACTIVITY AND EXOCYTOSIS IN GLUCAGON SECRETING RAT A-CELLS

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Patch-clamp techniques were used to explore the types of G proteins which mediate the inhibitory effects of somatostatin on glucagon secretion from single rat pancreatic A-cells. Somatostatin inhibited spontaneous electrical activity in the absence of glucose and repolarised the cell by >15 mV. Pre-treatment with pertussis toxin abolished the inhibitory response to somatostatin, indicating the involvement of an inhibitory G-protein. These effects on electrical activity were associated with an increase in the whole-cell K^+ conductance by 3.8±1.0 nS from a control level of 1.0±0.2 nS (n=4) at 25.6 mM $[K^+]_o$. In support for a direct interaction between G-proteins and the somatostatin-activated K^+-channel, intracellular application of GTPgS led to a slow but persistent activation of the current. This effect was mimicked by G-protein bg-subunits. Activation of the K^+ conductance by somatostatin was inhibited in cells treated with antisense oligonucleotides against G-proteins of the subtype G_{i2} but not G_{i1}, G_{i3} or G_o. Somatostatin reversibly inhibited depolarisation-induced increases in cell capacitance (used to monitor exocytosis) by >85% without affecting the whole-cell Ca^{2+}-current. This action was abolished in cells pretreated with pertussis toxin and the serine/threonine protein phosphatase calcineurin inhibitors deltamethrin and cyclosporin A. Finally, the effect of somatostatin on exocytosis was also prevented by antisense oligonucleotide treatment against G_{i2}- but not G_{i1}-, G_{i3}- or G_o-proteins. These data suggest that somatostatin inhibits glucagon release by G_{i2}-protein-dependent interaction with both proximal and distal inhibitory steps in the stimulus-secretion coupling of the rat A-cell.

OP 27
Nutrition and Diabetes

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EFFECTS OF DIETARY FAT ON INSULIN SENSITIVITY AND INSULIN SECRETION -THE KANWU STUDY.

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Experimental and clinical data indicate that the amount and quality of dietary fat may be of importance for development of insulin resistance and related metabolic disorders, but this has not been verified in interventional studies in humans.

Aims: To evaluate if a change of dietary fat quality affects insulin sensitivity and insulin secretion in humans.

Materials and methods: The study was performed simultaneously in five centres (Kuopio, Aarhus, Naples, Wollongong, Uppsala) and included 162 healthy men (n=86, mean age 48 years) and women (n=76, 49 years) who were randomised to receive controlled isoenergetic diets during three months - in a parallel single blind fashion - containing either a high proportion of saturated (SAFA diet) or monounsaturated (MUFA diet) fatty acids. The mean content of fat during the intervention was 38 energy percent (E%) in both diets, as evaluated from three days food records. The intake of saturated and monounsaturated fatty acids were 18 and 14 E% on SAFA and 10 and 22 E% on MUFA diet with 5 E% polyunsaturated fatty acids in both diets. Insulin sensitivity and insulin secretion were determined by the frequently sampled i.v. glucose tolerance test (The Minimal Model according to Bergman).

The results indicate that the dietary fat quality significantly influences the insulin sensitivity with an impairment on the SAFA diet (-10%, p=0.03) but no change on the MUFA diet (+2%, n.s.) with p=0.05 for the difference between the changes. Insulin release and glucose effectiveness remained unaffected. When total fat intake was taken into account the favourable effect of substituting MUFA for SAFA was lost in individuals consuming more than 38 E% fat.

Conclusion: This study demonstrates for the first time that insulin sensitivity is influenced by dietary fat quality, also in healthy humans. A change of the proportions of fatty acids in the diet, reducing SAFA and increasing MUFA, improves insulin sensitivity but has no effect on insulin secretion. This improvement is not seen in individuals with a high dietary fat intake.

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THE EFFECTS OF LONG-TERM SUPPLEMENTATION WITH PHARMACOLOGICAL DOSES OF VITAMIN E IN TYPE 1 DIABETIC PATIENTS ARE TEMPORARY, NOT SELECTIVE AND SATURABLE

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Aims: Vitamin E supplementation has been proposed as adjunctive therapy to counteract the increased low density lipoprotein oxidation seen in diabetes and thus prevent and/or delay cardiovascular complications. Clinical studies so far have been short-term and have varied widely as regards dosage, patient selection and metabolic control. In this study we proposed to investigate the impact of a moderate dose (750 IU/day) of vitamin E given for 1 year to metabolically stable type 1 diabetics. **Materials and Methods:** double blind randomisation into 2 groups: group S (n=22) received dl-α-tocopherol 250 IU 3 times daily for 1 year. Group P (n=22) received placebo for 6 months followed by α-tocopherol for a further 6 months. Metabolic control, serum vitamin E and lipoprotein peroxidability were monitored at inclusion, every 3 months and 3 months after stopping supplements. The evolution in time within each group and the between-groups effect of vitamin E vs placebo was analysed by repeated measures ANOVA. **Results:** Both groups were comparable at inclusion. Serum vitamin E doubled after 3 months of supplementation (from 15.9±4.7 to 28.6±7.9 μg/ml, M±SD, $p<0.0005$) but not under placebo. Although lipid profiles, HbA1c and blood biochemistry did not change, copper-induced in vitro peroxidability of LDL and VLDL decreased after 3 months of vitamin E ($p<0.005$ for both groups S and P) as indicated by a 30-60% decrease in the production of TBARS (thiobarbituric reactive substances) and prolongation of the lagtime for the appearance of fluorescent products (from 107±25 to 123±30 min in group S, p=0.002 vs placebo). Giving supplements for another 3-9 months did not change levels of serum vitamin E or lipoprotein peroxidability any further, but they returned to the inclusion values after stopping. Initial patient characteristics and serum vitamin E did not affect the subsequent antiperoxidative response to supplements, which was only related to the increase in serum vitamin E after 3 months ($r= -0.43$ $p=0.005$) but not later. **Conclusions:** Improvement in lipoprotein peroxidability of type 1 diabetics is saturable, limited to the period of vitamin E supplementation but independent of its duration or patient characteristics. The need for continuous maintenance supplements at a minimum effective dose should thus be considered in type 1 diabetic patients.

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DIABETES PREVENTION STUDY IN KUOPIO: ONE-YEAR INTERIM RESULTS ON DIET, WEIGHT AND INDICES OF GLUCOSE METABOLISM

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A controlled randomized lifestyle intervention for the prevention of type 2 diabetes in overweight subjects with impaired glucose tolerance was started in 1993 in five centers: Helsinki, Kuopio, Oulu, Tampere and Turku. **Aims**: To report the 1-year interim results on diet, weight and indices of glucose tolerance from Kuopio. **Materials and Methods**: Baseline characteristics (mean values) of the subjects were age 53 years and body mass index 31.2 kg/m^2. Baseline and 1-year measurements included weight, an oral glucose tolerance test and a 3-day food record. **Results**: At baseline the energy intake in the intervention group (n= 47, 19 males, 28 females) was 1917 kcal and in the control group (n= 47, 18 males, 29 females) 1789 kcal. The proportion of energy intake as fat, carbohydrates and protein was 35.2, 45.6 and 16.1 E% in the intervention group and 37.1, 44.6 and 16.3 E% in the control group, respectively, and there were no differences between the groups. In the intervention group there was a significantly greater reduction in energy intake after 1 year as compared to the control group (-294 kcal vs. -86 kcal, p= 0.04). In both groups there was a reduction in the proportion of energy from fat (-3.5 E% vs -3.7 E%, NS) and from saturated fat (-2.3 E% vs -1.7 E%, NS) but there was no difference between the groups. However, the total amount of saturated fat was lower in the intervention group after 1 year (11.2 vs 12.9 E%, p = 0.04). There was a significantly greater reduction in weight (-4.8 kg vs -1.4 kg, p <0.001), fasting /2-hour glucose (-0.29/ -1.27 vs 0/-0.28, p<0.04) and 2-hour insulin (-45.5 vs -19.4, p <0.04) in the intervention group when compared to the control group. **Conclusions**: A difference between the intervention and control groups was seen after 1 year in the reduction of energy intake, saturated fat intake, weight and main indicators of glucose metabolism.

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RELATIONSHIP OF GLUCOSE TOLERANCE STATUS TO RISK FACTOR CHANGES FOLLOWING ORLISTAT-INDUCED WEIGHT LOSS.

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Background: Type 2 diabetes (DM) greatly increases cardiovascular disease (CVD) risk factors but little is known about the capacity to improve risk in DM vs normal subjects (N). **Aim**: A pooled analysis of 3 randomized, placebo-controlled trials to evaluate changes in risk factors produced by weight loss with orlistat (ORL), a gastrointestinal lipase inhibitor which blocks absorption of ~30% of dietary fat, in obese adults who were N, DM, or impaired (IGT) at baseline. **Methods:** Subjects were randomized to double-blind treatment with orlistat 120 mg tid (n=359) or placebo (PLA, n=316) plus a hypocaloric diet. Oral glucose tolerance (OGT) was tested at baseline and after treatment. IGT was defined as 2-h serum glucose between 7.7-11.1 mmol/l and DM as 2-h glucose >11.1 mmol/L. **Results:**

	Normal		Impaired		Diabetic	
	ORL	PLA	ORL	PLA	ORL	PLA
Weight loss, %	-6.9±0.5	-4.0±0.4	-7.2±0.9	-3.4±0.9	-5.0±0.9	-3.8±1.2
LDL (%)	-9.8±0.4	-1.2±0.4	-8.2±0.5	-2.2±0.	-3.3±0.5	+7.3±0.4
Cholesterol	-7.9±0.5	-2.0±0.3	-7.8±0.4	-3.9±0.4	-3.6±0.4	+0.9±0.6
Tg (mmol/L)	-4.0±1.0	-3.1±0.8	-4.7±0.09	-4.2±1.0	-4.0±1.0	-16.7+5.
HDL	+0.9±0.2	+2.6±0.4	-0.5±0.2	-2.9±0.6	-3.9±0.5	-2.2±0.6
DBP (mm Hg)	-4.4±0.6	-2.1±0.6	-1.4±0.9	-1.8±1.6	+0.2±2.3	+0.1±3.1

ORL produced significantly greater weight loss and reductions in LDL and total-cholesterol than PLA for N, IGT and DM subjects (P<0.05). Changes in CVD were lower in DM subjects for ORL and PLA, due to the lower weight loss for this group. Changes in Tg were similar across treatments and OGT status. HDL was initially lower and decreased further in DM. **Conclusions**: These results highlight the benefit of ORL, compared to diet alone, in producing weight loss and improved CVD risk factors in obese subjects with varying degrees of glucose intolerance.

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Haemostasis

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Plasminogen Activator Inhibitor-1 synthesis is increased in arterial wall of diabetic subjects

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Aims: Plasma plasminogen activator inhibitor-1 (PAI-1) levels are increased in diabetes and this might contribute to decreased fibrinolysis and accelerated atherosclerosis in this condition. Arterial wall and in particular endothelial cells are among the tissues mainly responsible for PAI-1 synthesis and release into the circulation; elevated glucose concentrations increase PAI-1 synthesis in arterial wall cells in culture. However, arterial wall PAI-1 levels have not been investigated in diabetic patients. Therefore, aim of this study was to determine the effect of diabetes on PAI-1 levels in the arterial wall and on plasma fibrinolysis in humans. **Methods:** blood samples and a small tissue specimen from mammarian artery were obtained from 6 diabetic patients (blood glucose= 9.5±0.5 mM, age = 72±5 yrs, BMI= 25±3 Kg/mq) and 4 matched controls (blood glucose= 6.0±0.3 mM, age= 66±9 yrs, weight= 77±5 Kg, BMI 27±1 Kg/mq) who underwent coronary artery by-pass graft surgery. PAI-1 antigen localization in the arterial wall was obtained by immunoistochemistry and read by laser scannning confocal microscopy; plasma fibrinolytic activity was measured by lysis of fibrin plates. **Results:** PAI-1 related immunofluorescence was increased in the arterial wall of diabetic patients (1013±179 vs 652±67 arbitrary fluorescence units, p<0.01) while plasma fibrinolysis was sharply reduced in diabetic patients (mean lysis areas on fibrin plate= 525±58 vs 736±87 mmq, p<0.05). **Conclusions**:this data provide the first direct evidence that diabetes is associated with increased PAI-1 synthesis in the arterial wall. This suggests that in this disease endothelial cells can be an important source for increased plasma PAI-1 and that local fibrinolysis can be affected by increased PAI-1 levels in the arterial wall.

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INTERPLAY BETWEEN INSULIN AND CATECHOLAMINES ON PAI-1 GENE EXPRESSION IN HUMAN VISCERAL ADIPOSE TISSUE

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Enhanced production of type-1 plasminogen activator inhibitor (PAI-1) by visceral fat could partly be reponsible for the development of cardiovascular disease in central obesity. Unravelling the mechanisms involved in PAI-1 regulation are thus essential. We have previously shown that glucocorticoids stimulate and cAMP inhibit PAI-1 production by human visceral adipose tissue. The role of catecholamines has not yet been explored and the effects of insulin are still controversial. In the present study, we focused on the direct effects of catecholamines and insulin, and on the interplay between these 2 hormones. Visceral adipose tissue was obtained from patients undergoing elective abdominal surgery (n = 12, age: 58 ± 4 yr, BMI: 30 ± 3 kg/m^2; mean ± SEM), and explants were cultured in MEM for 8 - 24 h. Epinephrine, a mixed α- and β- adrenergic agonist, dose-dependently inhibited PAI-1 mRNA levels in adipose tissue. Isoproterenol (Iso), a pure β-adrenergic agonist was found to be more potent and a 55 - 60 % fall in PAI-1 mRNA and protein secretion was obtained at 10 µM. As expected, this inhibition was blocked by propanolol, a non-specific β-adrenergic antagonist, and was reproduced by isobutyl methylxanthine (IBMX), a phosphodiesterase inhibitor which indirectly elevates intracellular cAMP. Insulin (Ins) added alone to the medium had no or only a weak (+15 - 30%) stimulatory effect on PAI-1 mRNA and secretion. However, insulin partly reversed the inhibitory effect of isoproterenol (PAI-1 mRNA abundance: + 44% Ins + Iso vs Iso alone, p<0.05). Insulin also partly reversed the inhibitory effect of IBMX (+ 77% Ins + IBMX vs IBMX alone, p<0.05). In conclusion, insulin may stimulate PAI-1 gene expression in human visceral adipose tissue by a cAMP-dependent, β-adrenergic-independent mechanism. This effect is more readily detectable when tissue cAMP levels are elevated, which is consonant with the fact that some actions of insulin are brought about by decreases in intracellular cAMP in adipose tissue.

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VISCERAL FAT IS A DETERMINANT OF PAI-1 ACTIVITY IN DIABETIC AND NON DIABETIC OBESE WOMEN.

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Aims: PAI-1, an inhibitor of fibrinolysis and an important cardiovascular risk factor, has been shown to be elevated in obesity and type 2 diabetes. In the present study, we wanted to investigate the difference in PAI-1 activity between these two groups of patients and determine the role of visceral fat (VAT). Materials and Methods: PAI-1 activity (AU/ml) and visceral fat (CT-scan at level L4-L5) were measured in 2 groups of 30 patients matched for gender, BMI, fat mass, insulin, and subcutaneous abdominal fat. Results: VAT and PAI-1 activity were significantly higher in the obese diabetic women compared to the obese non-diabetic women ($p<0.001$;$p=0.007$) of similar body weight and body fat mass. In the whole group, VAT correlated significantly with PAI-1 activity, even after correction for insulin and triglycerides ($r=0.43$;$p=0.004$). Stepwise multiple regression analysis showed VAT as the most important determinant factor for PAI-1 in the whole group and in the group of obese non-diabetics. In the group of obese diabetics however, insulin was the most important determinant. Conclusions: These results show that visceral fat and not total body fat is the most important determinant of PAI-1 levels confirming results of previous in vitro studies. Furthermore, an increased amount of visceral fat in type 2 diabetics may contribute to the increase of PAI-1 activity levels and its subsequent risk for cardiovascular disease in diabetics.

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ACTIVATION OF PROTEIN KINASE C ISOENZYMES β1 AND δ BY THROMBIN IN HUMAN PLATELETS OF TYPE 2 DIABETIC AND NON-DIABETIC PATIENTS

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Aims: Protein kinase C (PKC) is involved in the development of diabetic complications such as macro- and microvascular changes. It is generally believed that signal transduction pathways are preactivated in diabetic patients. Therefore we tested the hypothesis whether PKC is more sensitive to stimulation in diabetic patients than in non-diabetic controls. **Materials and Methods:** Whole blood was acquired from age, weight and diseases matched patients (n=10) and controls (n=8). Platelets were purified by differential centrifugations and incubated with different doses of thrombin. Western-blots of PKC isoenzymes β1 and δ were performed with the protein homogenates of cytosolic and membranous fraction. The activation of PKC was determined by translocation from the cytosol to the membrane. To verify PKC activation we performed radioactive PKC-assays with the specific PKC substrate MBP_{4-14} with the same platelet homogenates. **Results:** Already after incubation with low doses of thrombin (0.5 and 1 U) we found significantly elevated protein levels of PKC β1 (2-3 –fold, $p<0,05$ with Wilcoxon test) in diabetic patients while the levels of control persons remained nearly unchanged. PKC δ was increased 1.5 -fold in the platelets' membranous fraction of diabetic and non-diabetic persons. Incubation with thrombin elevated PKC activity in diabetic persons to higher levels than in control persons. Additionally we found higher levels of active PKC in thrombin stimulated and unstimulated platelets of diabetic patients (2-fold) relative to an internal standard protein homogenate. **Conclusion:** Our results indicate that the calcium dependent PKC β in diabetic patients is hyperreactive whereas PKC δ remained nearly unchanged. The higher levels of active PKC after stimulation with thrombin and the higher sensitivity towards this protease in diabetic patients may be the consequence of a dysbalanced glucose and fatty acid metabolism. The changes in the PKC's activity may explain the influence on the platelets' altered release of pro-aggregatory metabolites such as thromboxane and may therefore play an important role in the mediation of macro- and microvascular dysfunctions.

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Macrovascular Disease

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Progressive Macrovascular Disease in a Population with Type 2 Diabetes

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Aims: To characterize the progression of macrovascular complications (MAC) with increasing duration of Type 2 diabetes (DM2).

Materials and Methods: All members of a large health maintenance organization with diagnosed DM2 between 1987-1997 were included in the study (n=16,287). Electronic and administrative records were used to stratify MAC into four stages: [1] *None*; [2] *At Risk:* hypercholesterolemia or hypertension; [3] *Diagnosed Disease:* angina, atherosclerosis, or PVD; [4] *Major Event:* Diagnosis of myocardial infarction, stroke, congestive heart failure, amputation, cardiac bypass surgery, or gangrene. Controls were members without diabetes, matched by age and gender. To determine the effect of diabetes on the progression of MAC, we used an ordinal log-likelihood model. We also modeled the effects of duration of diabetes and age at diagnosis on development of MAC among persons with DM2.

Results: Prevalence of MAC was significantly greater in the diabetic group compared to controls (68% vs. 32%, $p<0.001$). Most of the difference was accounted for by the *Major Event* (MajE) stage (29% vs. 14%, $p<0.001$) and the *At-Risk* stage (48% vs. 13%, $p<0.001$). DM2 independently increased the odds of experiencing a MajE by 2.7 (95% CI: 2.5-2.8). Duration of DM2 was associated with increased odds of experiencing a MajE, regardless of age at diagnosis. The relative prevalence of each stage remained stable for the first 10 years of diabetes. After 10 years' duration, the proportion at risk decreased ~3% while the proportion with MajE increased ~1% per year.

Conclusions: In this representative but aggressively treated population, MAC are twice as prevalent with DM2, and progression of MAC accelerates after a decade of diagnosed DM2.

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RISK FACTORS FOR CORONARY HEART DISEASE MORBIDITY AND MORTALITY DIFFER IN MEN AND WOMEN WITH TYPE 1 DIABETES

The EURODIAB Prospective Complications Study (PCS) Group
Royal Free and UCL Medical School, London, UK and 27 European Centres

Aims: To compare risk factors for coronary heart disease (CHD) morbidity and mortality in men and women with type 1 diabetes. **Materials and methods**: Data from 27 centres participating in the EURODIAB Prospective Complications Study, on type 1 patients aged between 15-60 years at baseline. Baseline CHD was defined as a doctor diagnosis of myocardial infarction, angina, or coronary artery bypass ₍raft (CABG), and probable or possible ischaemia on ECG. CHD status at follow-up was ascertained in those without CHD at baseline (920 men, 831 women). All blood and urine samples measuring baseline risk factors were assessed by a central laboratory. **Results** After a mean 7.3 years of follow-up, incidence of CHD was 3.9% (95% CI 2.8,5.4%) in men, and 4.3% (95% CI 3.1, 5.9%) in women. Significant risk factors at baseline, comparing those who did and those who did not develop CHD in men included age (41.9 vs 31.4 years, $p=0.0001$), HbA_{1c} (7.2 vs 6.6%, $p=0.04$), albumin excretion rate (AER) (38.3 vs 16.5 μg/min, $p=0.001$), cholesterol (5.5 vs 5.1 mmol/l, $p=0.03$) and LDL cholesterol (3.8 vs 3.3 mmol/l, $p=0.009$), all age adjusted. BP, triglyceride and HDL cholesterol were not significantly different. In contrast, for women, whilst age (41.5 vs 31.7 years, $p=0.0001$), HbA_{1c} (7.3 vs 6.6%, $p=0.03$), AER (37.8 vs 13.7 μg/min, $p=0.0001$), cholesterol (5.9 vs 5.4 mmol/l, $p=0.009$) and LDL cholesterol (3.7 vs 3.3 mmol/l, $p=0.04$) differed between those with and without CHD, further risk factors included systolic BP (126 vs 117 mmHg, $p=0.0002$), triglyceride (1.25 vs 0.89 mmol/l, $p=0.0001$), and presence of any retinopathy (76 vs 41%, $p=0.001$). **Conclusions:** We confirm the loss of female protection from CHD in the presence of type 1 diabetes. Risk factors for both men and women for CHD were glycaemic control, cholesterol, LDL cholesterol and AER. In addition for women, systolic blood pressure and triglyceride were also significant risk factors. These findings indicate further directions for research into the loss of the sex difference in CHD risk in diabetes.

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EARLY INCREASE OF OXIDATIVE STRESS IN IDDM PATIENTS WITH SHORT DURATION OF DISEASE: A SEX-RELATED DIFFERENCE

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Aim: The majority of epidemiological studies suggest that diabetes mellitus increases the risk of CHD to a greater extent in women than in men. IDDM patients show a reduced total plasma antioxidant capacity (TRAP) and augmented levels of total conjugated dienes (CD) and lipid hydroperoxides (ROOHs). Materials and Methods: We studied 37 IDDM patients attending our Diabetic Outpatients Clinic, with short duration of disease (6±3 yrs, range 1-10), without diabetic complications and with good metabolic control (7.4±1.2%) and 29 control subjects, compared for age, sex, smoking habit, diet as well as physical activity. Compared to diabetic men, IDDM diabetic women showed significative higher ROOHs levels (8.2±1.9 vs 6.4±2.2 mmol/l; $p<0.02$), a significative reduction of CD isomers Ratio (0.070±0.033 vs 0.99±0.036 [A.U.]; $p= 0.006$), 246 nm CD (0.0022±0.0011 vs 0.0032±0.0010 [A.U.]; $p=0.006$) and TRAP (579.8±95.4 vs 720.3±111.2 mmol/l; $p=0.0002$). No differences were found in control subjects. Significative difference was found for uric acid levels, the most important soluble antioxidant, between IDDM and controls (3.4±0.8 vs 3.8±0.9 mg/dl; $p=0.009$), between IDDM and control males (3.8±0.8 vs 4.4±0.8 mg/dl; $p=0.01$) and between IDDM and control females (2.8±0.6 vs 3.4±0.7 mg/dl; $p=0.01$). No significative differences were observed for age, duration of disease, BMI, metabolic control and lipid profile, between IDDM males and females In a multiple regression analysis significative relationship was found among TRAP, ROOHs and Ratio with sex ($P=0.009$; $P=0.027$; $P=0.034$, respectively) in IDDM patients.Conclusions: Many epidemiological studies suggest that the protective effect of female sex is loss in presence of diabetes mellitus. Our results suggest that IDDM women shown an early impairment of antioxidant status and an early increase in oxidative stress higher than IDDM men, without differences in glico-metabolic control. The early reduction of an antioxidant, such as uric acid, seems to contribute to this phenomenon.

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RELATIONSHIP BETWEEN POSTPRANDIAL GLUCOSE SPIKES AND INTIMA-MEDIA THICKNESS

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A recent report on the Diabetes Control and Complications Trial suggested that „the risk of complications may be more highly dependent on the extent of postprandial glycemic excursions" than on glycosylated hemoglobin. **Aim:** To analyse the relationship between postprandial glucose spikes (PGS) after an oral glucose tolerance test (OGTT) and the intima-media thickness (IMT) of the common carotid artery as a well-accepted marker of atherosclerosis. **Materials and methods:** Subjects (n=582) were examined from the RIAD Study (Risk factors in IGT for Atherosclerosis and Diabetes). Inclusion criteria: age 40-70 years, familial history of diabetes and/or obesity or hyperlipoproteinemia. Exclusion criteria: known diabetes, treatment affecting glucose tolerance. Carotid IMT was measured by high resolution B-mode ultrasound. Standard OGTT was conducted with 75 g glucose. Glycosylated hemoglobin was examined by high-performance liquid chromatography, plasma glucose at 0′, 30′, 60′, 90′ and 120′ after OGTT - by the hexokinase method. PGS were calculated as the difference between fasting and maximal postprandial glucose. **Results:** IMT_{mean} rose in quintiles of PGS: 0.83±0.02 mm (mean±SEM) in 1st quintile (Q; ≥ 0.46 and < 2.92 mmol/l); 0.83±0.02 mm in 2nd Q (≥ 2.92 and < 4.01 mmol/l); 0.87±0.02 mm in 3rd Q (≥ 4.01 and < 5.20 mmol/l); 0.88±0.02 mm in 4th Q (≥ 5.20 and < 6.80 mmol/l); 0.90±0.02 mm in 5th Q (≥ 6.80 and < 14.01 mmol/l) with a significant difference between 1st and 2nd Q vs. 4th and 5th Q. Similarly, a significant elevation was found for IMT_{max} in quintiles for PGS. These results were confirmed after adjustment for age, sex and HbA1c. PGS were significantly correlated to both IMT_{mean} and IMT_{max}, which remained significant after age, sex and HbA1c adjustment. Thus, our data suggest that PGS could be harmful for the endothelium and modify the risk for atherosclerosis.

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ENDOLUMINAL REVASCULARIZATION BY MECHANICAL ROTATIONAL ATHERECTOMY IN DIABETIC PATIENTS WITH LIMB THREATENING ISCHAEMIA.

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Aim: Limb threatening ischaemia for infrainguinal arterial occlusion is the principal cause of amputations in diabetes. Vascular reconstruction is performed mainly by distal by-passes with autologous saphenous vein. Mechanical atherectomy with Auth's rotational device appears to be a suitable technique for the treatment of distal arterial occlusions.Materials and methods: We studied 88 patients with type 2 diabetes (mean age 71±15 years, mean duration of the disease 19±12 years) that were referred to our department for revascularization or amputation because of ischaemic lesions of the foot. Digital subtraction angiography showed an occlusion of the femoral superficial artery in 24 patients, while popliteal and tibial arteries were occluded in 37 patients and significantly stenotic in 27 patients. Seven patients were not considered suitable for the treatment due to the length of the obstruction and/or the lack of a suitable pedal circulation. Mechanical atherectomy was performed in 81 patients without any significant side effect and resulted in immediate patency of the treated arteries. Results: In 77 patients vessels have been patent for a mean follow-up period of 55 months (range 2-76 months) with a limb salvage rate of 95%. One patient had occlusion of the treated segment after 3 months without recurrence of necrosis. Four patients were amputated, three did not improve because of a poor run-off of the pedal circulation and one had a severe infection. Conclusions: We conclude that mechanical atherectomy is a safe and effective endoluminal recanalization procedure in diabetic patients with limb threatening ischaemia due to infrainguinal

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Neuropathy-Risk Factors Predictors and End-Points

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CARDIOVASCULAR RISK FACTORS PREDICT DIABETIC PERIPHERAL NEUROPATHY IN TYPE 1 SUBJECTS IN EUROPE.

The EURODIAB Prospective Complications Study (PCS) Group
Royal Free and UCL Medical School, London, UK and 27 European Centres.

Aims: To examine the relationship between cardiovascular risk factors and the incidence of peripheral neuropathy (PN) in type 1 diabetic subjects from 27 centres participating in the EURODIAB Prospective Complications Study. **Materials and Methods:** PN was assessed at baseline and follow-up using a standardised protocol involving combinations of neuropathic symptoms, absent tendon reflexes, age related vibration perception threshold (VPT) and autonomic function test abnormalities (Ewing). Serum lipids/lipoproteins, HbA_{1c} and albumin excretion rate (AER) were measured in a central laboratory. **Results:** Of 1195 subjects with no PN at baseline (mean age 30.6 years; mean duration 12.4 years), 24.5% developed PN over the follow-up period (average 7.3 years). In those with no baseline abnormalities, 24.4% developed neuropathic symptoms, 19.5% had absent reflexes, 21.2% had abnormal VPT and 15.5% abnormal autonomic tests at follow-up. The incidence of PN was significantly positively associated with age, duration of diabetes and HbA_{1c} at baseline. After statistical adjustment for these 3 factors the following baseline variables were significantly predictive of the development of PN; BMI, AER, triglyceride ($p<0.001$), cholesterol and systolic BP ($p<0.01$). Retinopathy at baseline was more frequent in those developing PN (51%) than in those not (31%) ($p<0.001$). Those developing PN were significantly more likely to be current (31%) or ex-smokers (22%) than those who did not (26% and 16% respectively). **Conclusions:** This prospective study shows that over a 7-year period, about one quarter of type 1 diabetic patients will develop peripheral neuropathy, with age, duration of diabetes, and poor glycaemic control being major determinants. Cardiovascular risk factors such as serum lipids, BP, BMI, smoking and AER also significantly predict the development of PN, thus supporting the role of vascular factors in its pathogenesis.

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Elevated Lipid profile as a predictor of Neuropathy: The Sheffield Prospective Diabetes Study.

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A recent report has found an alarming association between diabetic neuropathy (DN) and markedly increased mortality. Despite being very common complication, the aetiology and potential risk factors of DN have not been clearly determined in a prospective study. **Aims:** The aim of Sheffield Prospective Diabetes Study was to identify the abnormalities of physiological, biochemical, haemorrhelogical and cellular function for complications of diabetes in type 1 diabetes. **Materials and Methods:** 66 newly diagnosed type 1 diabetic patients (mean age 31 ± 9(SD) duration (3 years ± 2) were identified and followed for 9 years. They had detailed neurological assessment (symptoms and signs score, nerve conduction, vibration perception threshold, warm thermal discrimination threshold and autonomic function tests) and blood samples taken for detailed biochemical and haemorrheological analysis at base line and at follow up. **Results:** At the 9 years follow up, 51 patients were studied of whom 22 were found to have DN using Dyck's criteria. As expected subjects with DN had significantly higher (p=0.001) mean HbA1 (11.6% vs 9.7%) compared to those without neuropathy (NN). In addition, the only biochemical parameters that were found to be risk factor for the development of neuropathy at baseline were raised levels of cholesterol (5.5 vs 4.7 mmol; p=0.01) and triglyceride (1.6 vs 1.3 mmol; p=0.04). **Conclusions:** This prospective study confirms the findings of recent large epidemiological studies linking cardiovascular risk factors to the development of DN, and perhaps suggest a vascular aetiology for DN. Improvement of potentially modifiable risk factors for neuropathy may be useful for the development of risk reduction strategies.

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CUTANEOUS ELECTROGASTROGRAPHY AND GASTRIC ELECTRICAL ACTIVITY IN TYPE 1 DIABETES MELLITUS.

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Aims : Gastro intestinal motor disorder is a frequent complication in diabetes mellitus. Cutaneous recordings of gastric electrical activity could be a valuable non invasive technique for recognising these abnormalities. **Patients and methods :** 69 type 1 diabetes mellitus patients (mean age : 42.4±11.8 years ; diabetes duration : 12.7±9.6 years) and 15 control subjects matched for age, sex and body mass index were studied. Diabetic patients were asymptomatic, free of complications and none took medical treatment apart from insulin. Cutaneous electrogastrography (EGG) was recorded during 4 h before and 4 h after a standard meal. Gastric electrical activity was evaluated by EGG spectral analysis and the parameters assessed were the percentile distribution of the 3 spectra of gastric slow-wave frequency : bradygastria for 0-2 cycles per minute (cpm), normogastria for 2-4 cpm and tachygastria for 4-10 cpm. A second EGG was performed 2 years later in 17 diabetic subjects. **Results :** The percentage of tachygastria was higher in diabetic patients than in control subjects in the pre, per and post prandial EGG, respectively 37.8±6.7 vs 26.4±8.8%, $p<10^{-3}$; 41.5±8.2 vs 23±10.5%, $p<10^{-3}$ and 38.1±7.3 vs 28.9±9.8%, $p<10^{-3}$. Diabetic subjects had a lower percentage of normal gastric slow waves in the entire period of recording (47.3±7.3 vs 63.2±11.1%, $p<10^{-3}$) and in pre (47.8±8.4 vs 60.4±9.6%, $p<10^{-3}$), per (46±10 vs 65.6±12.2%, $p<10^{-3}$), and post prandial (48.5±10 vs 61.1 vs 10.9%, $p<10^{-3}$) periods. Percentage of 2-to-4 cpm slow waves was correlated with patients' age ($r=0.254$, $p<0.05$), body mass index ($r=0.36$, $p<0.01$), HbA1c ($r=0.255$, $p<0.05$) and fructosamine ($r=0.354$, $p<0.01$). There was no significant difference between EGG performed at a 2 years interval. **Conclusions :** Gastro intestinal motor disorders are present even in asymptomatic diabetic patients. Increased presence of tachygastria is the predominant form of abnormal electrical activity. Hyperglycemia should be an important factor in the development of vagal nerve damage.

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^{123}I-MIBG RADIOAEROSOL AS A MARKER OF PULMONARY NEUROADRENERGIC FUNCTION : A VALIDATION STUDY IN DIABETICS.

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Aims: The pulmonary clearance of inhaled iodine-123 metaiodobenzylguanidine (^{123}I-MIBG) has been found to reflect the functional status of the neuroadrenergici system of the lungs in normal subjects. The aim of this study was to validate this technique in diabetic patients, model of functional denervation. Materials and Methods: we studied 10 patients with IDDM and autonomic neuropathy (AN) (mean age 53±7 yrs; mean duration 31±7yrs) compared to 10 IDDM patients without AN (mean age 40±9 yrs, mean duration 28±5yrs). We performed in all patients two scintigraphic studies by inhalation of 185 Mbq ^{123}I-MIBG and 740 Mbq ^{99m}Tc-DTPA respectively, the spirometry, the pulmonary diffusing capacity for carbon monoxide (DLCO) and a methacholine challenge test. Autonomic neuropathy was studied by cardiovascular autonomic tests. Results: no significative difference was found in ^{99m}Tc-DTPA clearance rate between the patients with and without AN(T1/2 90,8±13,6 min vs. 79,5±40,5 min). IDDM patients whit AN showed a faster ^{123}I-MIBG clearance rate in comparison with patients without AN (T1/2 110,5±18,6 min vs 141,1±17,3 min, $p<0,05$). No significant correlation was found in the whole sample study, between ^{123}I-MIBG and ^{99m}Tc-DTPA clearance rate ($r=0,25$,p=ns). All patients showed a normal respiratory functional pattern. Conclusion: the increased clearance of ^{123}I-MIBG in IDDM patients with AN may be attributed to a reduce uptake of the radiotracer by the adrenergic system. These data confirm that ^{123}I-MIBG radioaerosol can be considered as a useful marker of pulmonary neuroadrenergic fuction.

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INCREASED SERUM LEVELS OF SOLUBLE FAS (sFAS) IN TYPE 1 AND TYPE 2 DIABETIC PATIENTS WITH NEUROPATHY.

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The **aim** of this study was to investigate circulating sFas levels in the serum of type 1 diabetic patients and type 2 suddenly insulin-requiring diabetic patients, with or without diabetic complications. Recent studies indicated that Fas-mediated cellular apoptosis was involved in the pathogenesis and development of type 1 diabetes, now considered as an auto-immune disease. Serum levels of the soluble form of Fas receptor (sFas), probably generated by an alternative mRNA splicing or the proteolytic cleavage of the membrane receptor, was shown to be increased in several other auto-immune diseases, but at least to our knowledge, neither in type 1 or type 2 diabetes. **Materials and Methods**: we measured circulating sFas levels in the serum of healthy volunteers, type 1 diabetic patients and type 2 suddenly insulin-requiring diabetic patients, with or without complications, using a sFas specific ELISA. **Results:** serum levels of sFas in diabetic patients with vascular complications especially retinopathy (n=10) or without any complication (n=18) were not significantly different compared with healthy volunteers (n=8) (8.7 ±0.6 ng/ml and 9.1 ± 0.4 ng/ml vs 8.2 ± 0.6 ng/ml; NS). In contrast, serum levels of sFas in all diabetic patients with neuropathy (n=14) were significantly increased, not only compared to healthy volunteers (13.5 ± 0.9 ng/ml vs 8.2 ± 0.6 ng/ml; $p < 0.005$), but also to patients without complications (13.5 ± 0.9 ng/ml vs 9.1 ± 0.4 ng/ml; $p < 0.001$) or with vascular complications (13.5 ± 0.9 ng/ml vs 8.7 ± 0.6 ng/ml; $p < 0.001$) and that, independently of the type of diabetes. **Conclusion**: Recent works indicated that serum of type 1 and type 2 diabetic patients with neuropathy induced apoptosis in human and mice neuroblastoma cell lines. These studies concluded that neuropathy might be Fas-mediated.Then, our preliminary results suggest that a dysregulation of the Fas-system is involved in the pathogenesis of diabetic neuropathy which occurs with a similar frequency in type 1 and type 2 diabetic patients.

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Exercise and Diabetes

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IS THERE A DIRECT CONNECTION BETWEEN (VO2) max INCREASE AND INSULIN RESISTANCE DECREASE AFTER AEROBIC TRAINING

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Aims: To investigate the significance of connection between (VO2) max increase and insulin sensitivity after aerobic training program. **Materials and Methods:** In ten insulin resistant NIDDM 47.6±4.6 years old (group E) in the 15-day period there was applied a program of aerobic training (35min sessions of walking on treadmill, intensity 60.8±5.7 (VO2)max. frequency 5/wk and 1600 kcal diet. In the same time, an other ten insulin resistant NIDDN 45.9±5.5 years old (group C) were on 1600kcal diet. Before and after this period in both groups the following was measured: insulin sensitivity (M/I) by the method of hyperinsulin euglicemic clamp, and (VO2)max by Astrand loading test on ergocycle. **Results:** In contrast to the group C, in the second testing of the E group subjects, it was found a significant increase in M/I (2.42±1.06: 1.23±1.07 mg/kg/min/mU; $p<0.001$) and (VO2)max (29.16±5.01: 26.34±4.26 ml/kg/min; $p<0.005$). Variations of changes of (VO2)max ranged from -2.1% to 20.3%. In all the subjects of the group E it was found an increase in M/I (4%-264%), the individual levels of which was not always in the connection with a degree of (VO2)max changes. The coefficient of rank correlation was on the bound of significance (□=0.6; □=0.564 for p=0.05). **Conclusion:** The results have shown that the programmed aerobic training has a positive influence on insulin resistance in NIDDM, but the level of M/I increase is not always in the proportion with (VO2)max increase.

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LONG-TERM ENDURANCE TRAINING INDUCED CHANGES IN INSULIN SENSITIVITY, MUSCLE ENZYMES AND GLUT4 IN NIDDM PATIENTS.

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Aims: Adaptations of skeletal muscle are responsible for an important portion of the beneficial effect of exercise training on insulin sensitivity in NIDDM. The great majority of training intervention studies have applied training programs lasting from a few weeks to several months. Since this is considered a relatively short period in exercise physiology, we have recruited a group of well-controlled NIDDM patients (n=8) to participate in a two-year endurance training intervention. **Materials and Methods:** During the first year, the participants were intensively coached, the second year they were only periodically supervised. In this abstract, results from the first year are presented, together with some preliminary data of the second year. A hyperinsulinemic euglycemic clamp and an incremental bicycle ergometer test to exhaustion were performed at the start and after one year of training. Muscle biopsies were taken on the morning of the bicycle test for determination of citrate synthase (CS), 3-hydroxyacyl-CoA dehydrogenase (HAD) and GLUT4 protein content (Western blotting). Differences were tested for significance using two tailed paired t-tests with $p<0.05$. **Results:** Body weight and fat percentage did not change significantly as a result of training (from 83 ± 13 to 84 ± 12 kg. and from 29 ± 7 to 27 ± 5 % resp.). Glucose uptake ($mg \cdot kg^{-1} \cdot min^{-1}$) changed from 7.3 ± 1.6 to 9.5 ± 0.9 ($p<0.05$), maximal oxygen uptake ($ml \cdot min^{-1}$) from 2684 ± 634 to 3013 ± 508 ($p<0.05$). CS increased significantly from 0.38 ± 0.14 to 0.53 ± 0.21 and HAD from 4.37 ± 0.81 to 6.35 ± 1.09. HbA1c was unaltered after training (7.0 ± 1.7 vs. 7.1 ± 1.0). In concert with muscle enzymes, muscle GLUT4 protein increased clearly in the course of the training period. Preliminary data from the two year measurements show that the amount of time spend training decreased during the second year from 117 to 63 min per week. Muscle enzyme capacity as well as glucose uptake during clamp decreased accordingly. **Conclusions:** We conclude that long term endurance training can induce a large increase (30%) in insulin stimulated glucose uptake, and that this coincides with pronounced metabolic adaptations of skeletal muscle. However, preliminary data suggests that these adaptations wane when training activity falls below a (yet to be determined) certain minimum level.

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INTRAMYOCELLULAR LIPID CONTENT IS RAPIDLY REDUCED BY ONE BOUT OF SUBMAXIMAL EXERCISE

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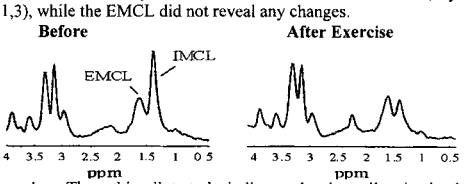
Proton magnetic resonance spectroscopy (MRS) studies revealed a distinct pool of lipids located within the skeletal muscle itself. These intramyocellular lipids (IMCL) have been found to be associated with insulin sensitivity and could thus play a role in the pathogenesis of type 2 diabetes. **Aims:** In order to get further insight how IMCL could be connected to the pathogenesis of insulin resistance, the regulation of this lipid compartment has to be understood. In the present pilot study, we addressed the question whether IMCL are acutely regulated by one single bout of endurance exercise. **Methods:** IMCL, extramyocellular (EMCL) and total muscle lipids (TML) of the tibialis anterior (TA) and the soleus muscle (SOL) were quantified by MRS in two well trained lean endurance athletes before and after a two hour run of about 60% VO2max. To achieve best reproducibility, we assessed different regions in TA and SOL and compared the individual spectra, the mean values and the difference spectra before and after exercise. **Results:** At baseline IMCL was within the normal range, while EMCL was slightly lower when compared to untrained subjects. After the run, IMCL had decreased markedly in both muscles, the TA and the SOL, by about 35 % (±21,3), while the EMCL did not reveal any changes.



Discussion: Thus, this pilot study indicates that in well trained subjects IMCL are metabolised during aerobic exercise. As indicated by the marked decline of IMCL, it seems that only the IMCL is acutely regulated, while EMCL is not. Thus, the intramyocellular lipid pool seems to be a rapidly regulated compartment.

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IN SITU ADIPOSE TISSUE LIPOLYSIS IN OBESE TYPE 2 DIABETIC PATIENTS SUBMITTED TO MODERATE PHYSICAL TRAINING

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Adipose tissue (AT) lipolysis is under control of catecholamines and of insulin which modulate the intracellular cAMP levels. Insulin acts as an antilipolytic hormone by activating a phosphodiesterase (PDE III) converting cAMP in AMP. We investigated 7 obese untrained type2 diabetic patients (age : 52.9±1.7 years) (mean±sem) submitted to a short term physical exercise program which consisted of cycling 40 min/day at 40 % of VO_2 peak, 5 days a week during 2 months. The same set of investigation was performed before and after 8 weeks of training. AT lipolysis was assessed using the in situ microdialysis technique: two 30 mm probes were inserted in the abdominal subcutaneous AT, and perfused with a Ringer solution supplemented with ethanol (to assess the local blood flow). After in vivo recovery test (0.5 µl/min), the perfusion flow rate was maintained at 3µl/min and 10-min fractions of the dialysate were collected. After 4 fractions, the first probe was perfused with increasing concentrations of noradrenaline (0.01, 0.1 and 1 µM), the second probe was perfused with 20 µM enoximone (PDE III inhibitor). Dialysate glycerol (lipolysis index) was measured using a radiometric enzymatic method. Ethanol outflow/inflow ratio was determined by an enzymatic method. Insulin sensitivity was assessed using euglycemic hyperinsulinemic (310mUI/l) clamp, the 2^d day of investigation. During the study, BMI and Hb A1c were stable (29.9 vs 29.8 kg/m^2, 6.44 vs 6.38 %). Whole body glucose disposal was improved (6.34±0.51 vs 7.39±0.35 kg/kg/min, P<0.05). Basal extracellular glycerol levels in AT decreased (229±22 vs 171±17 µM, P<0 05) without any change in basal blood flow. The in situ enoximone perfusion increased extracellular glycerol (128±10 % of baseline) before the training period, this effect was improved (157±17 %, P<0.05) at the end of the study. The in situ lipolytic effects of noradrenaline were not modified (219±29 vs 201±19 % of baseline at 1 µM). The effects of noradrenaline and of enoximone on local blood flow were not modified. Moderate short term physical training improving insulin sensitivity decreases AT basal lipolysis, increases the antilipolytic tone of PDE III in adipocytes without changes in cathecholamines-induced lipolysis in type 2 diabetics.

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METFORMIN INTERACTS WITH TRAINING TO LOWER GLYCEMIA AND TO INCREASE GLYCOGEN STORES IN DIABETIC RATS.

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Aims: Like in humans, lower amounts of glycogen (GLY) are present in tissues of diabetic rats. However, some improvements in the metabolic control can be obtained by training or drugs that lower glycemia. Metformin (MET), increased GLY while decreased the glycemia in normal rats stressed by exercise. The aim of this work was investigate if regular exercise and MET effects may interact to improve the metabolic control of diabetic rats. **Materials and Methods:** Alloxan diabetic Wistar rats, 3 month old, treated with MET (DTM) or not (DT), swam during 30 min in a tank with water at 30 ± 2°C, with 3% of their weight attached to the body. Training consisted of 20 sessions of 30 min, 5 days a week. Rats were adapted to water and to the exercise before rendered diabetic. Sedentary diabetic rats served as control (D and DM). MET (2.2 mg/kg/d) was given in the drinking water. The samples were taken after 24 h resting, under anesthesia (pentobarbital 40 mg/kg, *ip*). Glucose (mg/dL) was measured in plasma and GLY (mg/100 mg of wet tissue) in liver, soleus and gastrocnemius. Two-ways ANOVA were used to access significance (α<0.05). Table shows the **Results** (mean ± SD, N = 4).Significance: # p<0,05 versus D, ♦ p<0,05 versus DT).

Groups	Liver	Soleus	Gastrocnemius	Glycemia
D	1,29 ± 0,04	0,18 ± 0,01	0,2 ± 0,03	461,2 ± 83,5
DM	3,41 ± 0,06#	0,34 ± 0,01#♦	0.36 ± 0,03#	180,1 ±14,7#
DT	3,32 ± 0,51#	0,23 ± 0,05	0,52 ± 0,09#	144,2 ± 12,4#
DTM	6,61 ± 0,91#♦	0,42 ± 0,16#♦	0,6 ± 0,03#	138,8 ± 19,8#

Conclusion: Very small doses of oral metformin, alone, partially restored glycemia. The association between MET and training was beneficial, lowering glycemia further while increasing the GLY content of diabetic rats.

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Clinical Aspects of Insulin Action

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NORMOGLYCAEMIC WOMEN WITH PREVIOUS GESTATIONAL DIABETES HAVE DECREASED POSTPRANDIAL ENERGY EXPENDITURE

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Aims: Defective energy balance may represent a common pathogenic mechanism in the development of obesity and obesity-related metabolic disorders. To investigate the role of abnormal energy balance in the pathogenesis of type 2 diabetes, we studied women with previous gestational diabetes (GDM), a group known to be at high future risk of diabetes. Materials and methods: Resting energy expenditure (REE) was measured by continuous indirect calorimetry and postprandial thermogenesis (PPT) was assessed for 2 hours following a mixed meal (calculated to provide 42 KJ/Kg lean body mass). The subjects studied were all European and normoglycaemic at the time of study: 13 had a history of previous GDM and 23 were controls (normal glucose homeostasis during and after pregnancy). The two groups were matched for age, parity and time since delivery. Results: Waist:hip ratio, BMI, fasting leptin, fasting glucose and lipids were similar in the 2 groups. There was no difference in REE and REE/lean body mass between the 2 groups (median (IQR), 5514 (5086-5831) vs 5639 (5352-6195) KJ/24h, P=0.4; 65 (62-71) vs 71 (63-76), P=0.3, KJ/24h/kg of lean body mass; respectively). However, compared to control women, women with previous GDM had significantly reduced PPT (calculated as the incremental area under the curve: 83 (75-93) vs 101 (82-126) KJ, P=0.02). This remained significant when reanalysis included BMI as a covariate (P=0.04). There were no differences in fasting non-esterified fatty acids (NEFA) (448(334-547) µmol/l vs 476(301-643) µmol/l, P=0.5) nor in NEFA suppression during the test between the 2 groups. Conclusions: We have confirmed and extended previous findings demonstrating that there is a reduction in postprandial thermogenesis in normoglycaemic women with previous GDM. We conclude that reduced capacity for energy dissipation represents one mechanism by which these women are predisposed to obesity and type 2 diabetes.

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DAY-NIGHT VARIATIONS IN GLUCOSE METABOLISM ARE ASSOCIATED TO CONCOMITANT CHANGES IN LIPID METABOLISM IN TYPE 2 DIABETES MELLITUS.

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The occurrence of associated and possibly related day-night variations of glucose and lipid metabolism has been suggested but never demonstrated in patients with type 2 diabetes mellitus (T2DM). We designed the present study to assess the existence of day-night variations in glucose and lipid metabolism in T2DM. For this purpose, 9 diet-treated type 2 diabetic patients (6M, 3F; age 54±2 years; BMI 27.8±0.8 kg/m^2) were examined with a combination of 3-hour isoglycemic-hyperinsulinemic (10 mU/m^2/min) clamp and isotopic (6,6-$^2H_2$-glucose, hot-ginf; 1,1,2,3,3-$^2H_5$-glycerol) techniques. All patients were studied on two occasions, a week apart, between 0500 and 0800 h (study AM) or between 1700 and 2000 h (study PM), after the same interval of fasting (9 hours). Before clamp studies, plasma glucose (PG) and insulin (PI) concentrations were greater AM vs PM (PG, 6.7±0.3 vs 5.3±0.2 mmol/L, p<0.01; PI, 126±18 vs 98±6 pmol/L, p<0.05), whereas plasma free fatty acid (FFA) but not glycerol (G) values (FFA, 0.42±0.06 vs 0.80±0.06 mmol/L, p<0.05; G, 105±7 and 111±3 µmol/L, p=NS) were lower AM vs PM. During clamp, at steady-state PG (5.2±0.2 mmol/L) and PI (206±13 pmol/L), systemic glucose production was greater AM vs PM (5.61±1.20 vs 0.84±0.59 µmol·kg^{-1}·min^{-1}, p<0.01) and associated to increased G concentration and appearance (GA), (G, 82±7 vs 70±7 µmol/L; GA 2.21±0.19 vs 1.83±0.15 µmol·kg^{-1}·min^{-1}, both p<0.05). Glucose utilization rates AM and PM were not different. Thus, T2DM patients exhibit associated diurnal variations of glucose and lipid metabolism. In T2DM, insulin resistance at liver (and kidney) and adipose tissue is greater AM vs PM. However, the greater lipolysis AM is masked by hyperinsulinemia and hyperglycemia stimulated re-esterification of FFA.

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MEASUREMENTS OF INTERSTITIAL MUSCLE INSULIN AND GLUCOSE IN TYPE II DIABETIC SUBJECTS

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Aims: To measure interstitial muscle insulin and glucose in patients with type II diabetes in order to evaluate whether deficient transcapillary transport contributes to the peripheral insulin resistance. **Materials and methods:** 10 type II diabetes patients and 10 healthy controls (f-glucose 12.4 ± 1.0 vs. 5.4 ± 0.1 mmol/L, p<0.001, f-insulin 11.1 ± 2.9 vs. 5,6 ± 0,9 mU/L, ns) matched for sex, age and BMI were investigated. Plasma (arterialized venous) and interstitial insulin and glucose (measured by intramuscular microdialysis) in the medial quadriceps femoris muscle were analysed during a hyperinsulinemic euglycemic clamp. Blood flow in the contralateral calf was measured by strain gauge pletysmography. **Results:** At steady state clamping, the interstitial insulin concentration was significantly lower than plasma insulin in both groups (57 ± 12 vs. 149 ± 14 mU/L, p< 0.01, in controls and 81 ± 23 vs. 175 ± 11 mU/L, p<0,05, in diabetic subjects, respectively) and did not differ significantly between diabetic subjects and controls. The plasma-interstitial glucose concentrations differences were also similar in the two groups (2.4 ± 0.2 vs. 2.4 ± 0.4, ns, in controls and diabetics, respectively). Leg blood flow was significantly higher in controls (8.1 ± 1.2 vs. 4.4 ± 1.2 ml/100 g/min, p<0.05). The calculated muscle glucose uptake was less in diabetic patients compared to controls (4.2 ± 0.7 vs 6.5 ± 0.7 umol/100 g/min, p<0.05, respectively) and correlated significantly with glucose infusion rate (r^2 = 0.341, p<0.05) while there was no correlation between estimated muscle insulin uptake and glucose infusion rate (r^2 = 0.116, ns). **Conclusions:** During hyperinsulinemia, muscle interstitial insulin and glucose concentrations were similar in patients with type II diabetes and healthy controls despite a significantly lower leg blood flow in diabetic subjects. The data suggest that 1) neither insulin nor glucose concentrations in the interstitial fluid of human skeletal muscle is regulated by blood flow and 2) the decreased muscle glucose uptake in type II diabetes is caused by insulin resistance at the cellular level and not by a deficient delivery of insulin and glucose.

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LIPOLYSIS IN SKELETAL MUSCLE IS ACUTELY REGULATED BY LOW PHYSIOLOGICAL DOSES OF INSULIN

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In the pathogenesis of an impairment of lipid metabolism plays an important role. Patients with type 2 diabetes and normoglycemic insulin resistant subjects are characterised by an augmented muscular lipid content. **Aims and methods:** To see whether lipolysis (LL) of these muscular lipids is regulated by low physiological levels of insulin, we applied the microdialysis technique (MD) to 19 healthy subjects (12m/7f), with a mean age of 27.4years and BMI of 24kg/m2 to assess tissue metabolites during a 3-step euglycemic clamp (I=0.1, II=0.25 and III=1.0mU/kg*min). Two double lumen MD catheters (CMA60) were inserted each in the paraumbilical subcutaneous adipose tissue (AT) and in the tibialis anterior muscle (TA) to dialyse interstitial fluid (ISF), in which Glycerol (an index of LL) and tissue flow (ethanol outflow) were measured. **Results:** Serum FFA were lowered to 60%(I), 22%(II) and 7%(III)of basal. While serum Glyc decreased to 69%(I), 55%(II) and to 48%(III), Glyc-AT was reduced to 81%(I), 55(II) and decreased further to 25% of basal at III. Glyc-TA declined to 73% (I) and to 57% (II) but remained unchanged at III. Glucose infusion rate of exogenous glucose was 0 at I, 2.1 at II and averaged 8.9(mg/kg*min) at III. Tissue flow was higher in TA and remained unchanged in both compartments throughout the experiment. Suppression of LL in TA was most pronounced at the lowest levels of insulin. **Conclusion:** The data indicate that LL in both tissues is regulated at very low physiological concentrations of insulin, as indicated by the pronounced reduction of Glyc in both tissues (I and II). Higher insulin levels do not further reduce LL in skeletal muscle, hence, there is still a substantial amount of Glyc release in TA, indicating either a relevant LL or generation of Glyc by other sources. Thus, the present study suggests that LL of muscular lipids is tightly regulated by insulin.

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CHRONIC L-ARGININE THERAPY IMPROVES INSULIN SENSITIVITY IN NIDDM PATIENTS

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Background: Recent studies have shown that nitric oxide-mediated vasodilator response is blunted in NIDDM patients and that insulin-mediated nitric oxide vasodilation is impaired in these patients. However, little is known about the possibility that an increase in nitric oxide availability could increase insulin sensitivity in NIDDM patients. **Aims:** To evaluate the effects of a chronic administration of L-arginine, a precursor of nitric oxide synthesis, on peripheral and hepatic insulin sensitivity. **Materials and Methods:** Six lean NIDDM patients (age 59±5 yrs, BMI: 25.4±0.3 kg/m^2) in good metabolic control (HbA1c: 5.7±0.4%) treated with diet alone were studied. The study design consisted in a single blind study for a total duration of 3 months: in the first month patients were treated with the usual diet, in the second month patients were treated with diet+placebo (three times/day, orally) and in the third month patients were treated with diet+L-arginine (3 grams x 3/day, orally). At the end of placebo and L-arginine therapy, patients underwent a euglycemic hyperinsulinemic clamp (e.c.; 25 mU/kg/h) combined to 6.6-$^2H_2$-glucose infusion lasting two hours for the evaluation of hepatic glucose production (HGP). **Results:** Body weight, HbA1c, fructosamine, basal glucose and insulin levels were similar during the three periods. After L-arginine treatment, systolic blood pressure decreased by 8%, forearm blood flow increased by 36% ($p<0.02$). During e.c., M value (index of peripheral insulin sensitivity) increased by 34% ($p<0.002$) and HGP decreased by 29% ($p<0.05$) compared to placebo. Cyclic GMP, second messanger of nitric oxide significantly increased by 61% ($p<0.05$). **Conclusions:** Chronic L-arginine therapy significantly improves peripheral and hepatic insulin sensitivity in NIDDM patients without affecting insulin levels.

OP 33
Health Care Delivery

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Depression Prevalence, Treatment, and Costs of Health Care in Type 2 Diabetes

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Aims: To estimate depression prevalence in a large population-based sample of patients with type 2 diabetes in comparison to an age and gender matched control group; to compare pharmacological treatment of diabetes of those depressed with those not depressed; to compare annual, incremental, and aggregate health care costs.
Materials and Methods: We used age and gender to match 5059 patients with type 2 diabetes to a non-DM control group and then used electronic medical and pharmacy records to compare depression prevalence and annual, incremental, and aggregate health care costs.
Results: Consistent with smaller studies, we found depression to be much more prevalent in patients with diabetes than in control patients (18.5% vs. 11.4%, $p<.001$). In both groups, depressed patients were younger, were more likely to be female, and had more comorbidities. Within the diabetes group, depressed patients were more likely to be using insulin (36.2% vs. 24.5%, $p<.001$). Depressed patients in both the diabetes and control groups were 1.6 and 2.0 times more costly, respectively, than non-depressed patients ($p<.001$). However, aggregate incremental costs associated with depression were 2.3 times greater in the diabetes group than in the control group ($p<.001$).
Conclusions: The dollar values of the additional costs associated with depression were similar for both groups, suggesting that depression may add a fixed amount of cost regardless of diabetes status. However, the greater prevalence of depression in diabetes produced much greater aggregate costs in the diabetes population.

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TELEMATICS IN DIABETES CARE: APPLICATION OF AN ADVISORY PROGRAM BASED ON ROUTINELY MONITORED SELF-CONTROL DATA

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Aim: To bridge the gap between out-patient and in-patient care sectors, a telematic-based system was designed to provide the patient with required advice independent of time and actual location. **Material and Methods:** The network-based system TeleDIAB^R was established, comprising two main components: (1) the interactive advisory program KADIS^R (Karlsburg Diabetes Management System), located at a diabetes centre, and (2) a bi-directional telemedicine service to provide the centre with home-monitored self-control data (SCD) and to transmit to the patient any advice required in metabolic management. KADIS^R comprises a problem-oriented knowledge base and tools for the individual adaptation of the program to routinely monitored SCD and for the prediction of effects of defined therapeutic measures on the 24h-blood glucose profile. Two studies were performed to validate the entire program: a short-term (3 to 4 weeks) feasibility study (FS) to verify the adaptation function of KADIS^R to individual SCD sets, and a long-term (up to 15 months) application study (AS) to look after potential effects of system application in diabetes care. FS was performed during a summer camp in 43 diabetic children (age: 8 - 18 y). Between 61 and 337 SCD sets were monitored and transmitted to the diabetes centre for final evaluation. AS was carried out in 14 diabetic patients (age: 46 - 82 y) during routinely treatment by a G.P.. After successful adaptation of the program to the SCD sets, up to six KADIS-supported interactive advisory trails were performed in each patient on the occasion of routinely visits. $HbA1_C$ was followed over up to 15 months. **Results:** In FS 41 out of the 43 patients looked at, KADIS^R could be successfully adapted to SCD, for the remaining two patients, the system identified a lack of data and recommended to monitor SCD on three additional days. Thereafter, KADIS^R was also successfully adapted. In AS, $HbA1_C$ at the beginning was 9.3 $\pm$ 1.3 %. During KADIS^R-supported advice, $HbA1_C$ continuously decreased to 6.8 $\pm$ 0.5 % ($p<0.01$, follow-up time 7.9$\pm$3.5 months). **Conclusion:** Network-based telemedicine service in combination with interactive advisory program may provide an efficient tool to improve efficacy of diabetes care. The only data base required to run this system, is routinely monitored self-control data.

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QUALITY OF METABOLIC CONTROL IN AN INTERNATIONAL STUDY OF ADOLESCENTS WITH TYPE 1 DIABETES OVER 3 YEARS.

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From the total number of 2873 children and adolescents included in an international study in 1995 , 892 adolescents were restudied in 1998 including clinical and demographic data and HbA_{1c} centrally determined both years. **Aims:** To investigate differences in blood glucose control among centres in the 3 year period. **Material and methods:** The young people attended 21 paediatric departments in 17 countries in Europe, Japan and North America. There were 441 boys and 451 girls, mean age 11.3 ± 2.2 years and mean diabetes duration 4.6 ± 3.0 years in 1995. Only children with a diabetes duration of one year or more (1995) were considered thus excluding most children in the remission phase. All were treated by the same departments during the study period. **Results:** The grand mean for HbA_{1c} was 8.9 ± 1.6% which was similar to the average HbA_{1c} in 1995 for children aged 11-18 years (8.9 ± 1.8%). On average 32 % of the children had HbA_{1c} values equal to or below 8%. The incidence of hypoglycaemia resulting in unconsciousness and /or seizures was 18.4/100 patient years which was comparable with the incidence in 1995 for the same age group: 17.6/100 patient years. As in the 1995 study HbA_{1c} varied significantly between centres (range 7.6± 1.2 % - 11.0 ± 1.7%). A comparison of all HbA_{1c} values for the two years showed that 4 centres had improved their glycaemic control significantly ($p<0.05$) while none of the centres had deteriorated statistically significant in HbA_{1c} concentration. The 1995-1998 difference for the 4 centres with significantly improved HbA_{1c} values were not readily explicable in terms of insulindose or number of daily insulin injections. This indicates that other factors than insulin may be of importance for a satisfactory blood glucose control. Interestingly, the six centres of excellence in 1995 all maintained their level of good metabolic control in 1998 and even had a lower rate of hypoglycaemic events than the other centres. **Conclusions:**To obtain a better quality of care there is a need to set up new international clinical standards and criteria for childhood diabetes.

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RESULTS OF THE GERMAN IMPLEMENTATION OF THE EU-PROJECT DIABCARE QUALITY NETWORK IN EUROPE BY DIABCARE BAVARIA

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Since January 1997 DIABCARE Bavaria offers Germanwide health care providers to use the DIABCARE Fax System as a tool of quality assessment. The system allows the automatic aggregation and anonymized evaluations of diabetes data-sets on DIABCARE standard in benchmarking graphs. So far 119 institutions were registered for participation in the project office of DIABCARE Bavaria and 6.801 data sets have been aggregated. Evaluations of process indicators showed enormous differences between the participating institutions, e.g. the performance of the examination of feet within the last 12 months. Altogether foot examinations within the last 12 months were performed by 78% ± 20% ($\bar{x}$ ± SD)of the patients. 21% of the institutions documented the performance of foot examinations by less than 50% of their patients, on the other hand 25% of the participants performed examination by more than 90% of their patients. The comparison of 1.083 data sets documented in 1997 and 1.142 data sets documented in 1998 of 4 institutions from different health care levels (university clinic(1), hospital with specialisation in diabetes (2), GP without spezialisation on diabetes (3), GP with specialisation on diabetes(4)) showed in average an improvement of process indicators as performance of foot, eye and microalbuminuria (MAU) examination. The potential of center-external evaluations were used only by

	1		2		3		4	
	1997	1998	1997	1998	1997	1998	1997	1998
n (data sets)	303	263	612	707	116	63	52	109
% Feet exam. performed	86 %	90 %	88 %	87 %	76 %	90 %	82 %	84 %
% Eye exam. performed	66 %	71 %	74 %	71 %	27 %	35 %	25 %	61 %
% MAU-documentation	81 %	95 %	68 %	96 %	50 %	35 %	82 %	72 %

1/3 of the users (280 requests since 1997). It is concluded that medical doctors need urgently quality management training.

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THE UK DIABETES, INFORMATION ANALYSIS AND BENCHMARKING SERVICE (UKDIABS)

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Aims To determine if data routinely collected in diabetes information systems (DIS) can be analysed robustly to provide reliable benchmarks of clinical diabetes services 'performance'

Methods Data were collected from 37 diabetes information systems throughout the UK. For all patients with a recorded clinical entry (reviewed) during 1997 (102,979 records from a total cohort of 145, 803 patients) the UK diabetes dataset was extracted. Process benchmarks were calculated as the percentage of those reviewed who had the completed process (e.g. dilated fundoscopy) recorded; target benchmarks as the percentage of those measured who achieved the target (e.g. BP<160/90); and complication rates as the percentage of those reviewed with the recorded complication (e.g. diabetes-related amputation).

ResultsThere were wide variations in all measures. For example BP checks varied from 33% to 98% (mean 71%); Systolic BP < 140 mmHg. from 28% to 43% (38%), <160 mmHg from 61 to 85% (72%); Glycated haemoglobin from 17% to 99% (70%); serum creatinine > 250μmol. L^{-1} from 0.1% to 3.0% (1.1%); and prevalence of lower limb amputations from 0.25% to 2.5% (1.0%). 4911 people had had a previous MI or CVA (4.8%). Of these cholesterol was measured in 49%, >5.2 mmol/L in 24% and < 5.2 mmol/L in only 27%. More than 30% of patients had not had any form of diabetes review; of those who did only 43% had weight, BP and glycaemic checks with only 21% also receiving foot and eye examinations.

Conclusions It is possible to aggregate data routinely collected during clinical care. These results suggest that the majority of patients do not receive even a basic structured annual review.

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The Role of Leptin in Insulin Secretion

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LEPTIN INHIBITS MOUSE PANCREATIC ISLET CELL PROLIFERATION

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Aims. A functional leptin receptor has been found on the B-cells, and several reports indicate that leptin inhibits insulin secretion. We have now studied the effect of leptin on islet cell proliferation in obese hyperglycemic mice. These mice lack functional leptin, and they have up to ten times larger pancreatic islets than their lean littermates. **Methods.** From the age of 20 days, the mice were injected daily with 0.5, 1.1 and 3.0 mg leptin/kg for six days. Islet cell proliferation was measured as BrdU labeling index in vivo at the end of this period. **Results.** Food intake, body weight, serum insulin, and blood glucose decreased in a dose dependent manner. Islet cell proliferation rate decreased from 2.2% in obese controls to 1.2% and 0.4% in mice injected with 0.5 and 3mg leptin/kg respectively. When mice treated with 1.1 mg leptin/kg were pair fed with obese controls not receiving leptin, no differences were seen in weight gain and blood glucose levels. Islet cell proliferation were 1.8% and 0.6% in pairfed controls and leptin treated mice respectively. Serum insulin was also decreased. When isolated islets were cultured for two days with 62.5 nM leptin, the islet cell proliferation rate was decreased (2.0% versus 1.1% for controls). When lean mice were treated with 3 mg leptin/kg daily, the islet cell proliferation was lower than in lean controls (0.4% versus 1.0%). $P<0.05$ for all comparisons using Student's *t* test. **Conclusions.** The results suggest that leptin inhibits islet cell proliferation in both lean and obese hyperglycemic mice, and that this effect, at least in part is exerted directly on the islets. An inhibitory effect of leptin on islet cell proliferation is consistent with the phenotype of the obese hyperglycemic syndrome, and suggests an additional mechanism for the linkage between obesity and type 2 diabetes.

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TUMOR NECROSIS FACTOR (TNFα) AND LEPTIN EFFECTS ON INSULIN TRANSCRIPTION AND SECRETION IN HIT-T15 PANCREATIC CELLS

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Aims: TNF-α, a cytokine produced at inflammatory sites and in adipose tissue, is known primarily for its detrimental effects on insulin action. There is evidence to suggest that TNF-α may also influence β-cell function. Leptin is another adipose tissue derived hormone that might also act on β-cells. We explored the independent and combined effects of TNF-α and leptin, upon insulin transcription and secretion in the HIT-T15 pancreatic beta cell line.

Materials and Methods: HIT-T15 cells (passages 66-70) were cultured in F-12K medium for 40 hours in the presence of basal (7mM) and high (16.7mM) glucose, and 50ng/ml of TNF-α, or 50ng/ml of leptin or both together. Insulin secretion was measured by RIA. Insulin mRNA levels were evaluated by a semi-quantitative RT-PCR method, after normalization with β-actin mRNA, analyzed by an Image Analyzer system and expressed as integrated optical density units.

Results: TNF-α suppressed significantly insulin secretion at both basal and high glucose concentrations (TABLE). In addition, TNF-α significantly suppressed insulin mRNA levels, an effect that was more powerful in the presence of high glucose. Leptin also appeared to inhibit insulin mRNA and protein levels, but did not potentiate the TNF-α effects (TABLE).

	Control	TNFα	leptin	TNFα +leptin
Insulin levels (7mM)	154 ± 5	128 ± 6*	141 ± 3	125 ± 3*
Insulin levels (16.7mM)	167 ± 5	136 ± 5*	150 ± 11	126 ± 4*
Insulin mRNA (7mM)	147 ± 37	103 ± 4	122 ± 16	126 ± 14
Insulin mRNA (16.7mM)	192 ± 20	75 ± 5*	113 ± 13*	103 ± 14*

Values are mean ± SE, insulin levels are in μU/ml, *: $p < 0,05$ vs. control

Conclusions: TNF-α suppresses both insulin transcription and secretion in HIT-T15 cells, an effect that is enhanced significantly by high glucose but is not altered by leptin. This might contribute to the abnormalities of glucose metabolism that characterize conditions of increased TNFα production.

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DIFFERENTIAL EFFECTS OF TUMOR NECROSIS FACTOR (TNF-α) AND LEPTIN ON INSULIN SECRETION IN RIN-m PANCREATIC CELLS.

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Aims: TNF-α, a cytokine with important effects on glucose and lipid metabolism, is released in the circulation in response to infectious / inflammatory stimuli and in conditions of cancer cachexia. TNF-α is also overproduced, together with leptin, in the adipose tissue of obese individuals. We examined the acute and chronic effects of TNF-α and leptin on β-cell function in RIN-m rat pancreatic cells.

Materials and Methods: Cells (passages 19 - 22) were cultured in the presence of 11 mM glucose (RPMI 1640 medium) and two different concentrations of TNF-α (10 and 100 ng/ml) and leptin (15 and 50 ng/ml), for 1, 3, 8, 18, 27, 52 hours, respectively. Insulin levels from each culture supernatant was measured by RIA.

Results: In the presence of both TNF-α concentrations, insulin levels were significantly suppressed at 27 hours and remained suppressed by 52 hours. In contrast, leptin (50ng/ml) stimulated insulin secretion, an effect that also appeared at 27 hours and persisted up to 52 hours. Exposure to 15 ng/ml of leptin had no appreciable effects on insulin levels at any time point. (Table). As RIN-m cell line consists of beta and delta cells, we examined whether changes in somatostatin expression might contribute to these effects of TNF-α or leptin. Thus, we measured somatostatin mRNA levels by semiquantitative RT-PCR analysis after normalization against GAPDH mRNA, and found that TNF-α appears to increase, while leptin seems to diminish somatostatin mRNA levels.

	TNF-α control	TNF-α 10ng/ml	TNF-α 100ng/ml	Leptin control	Leptin 15ng/ml	Leptin 50ng/ml
3 hours	72 ± 3	76 ± 3	73 ± 1	65 ± 4	67 ± 3	65 ± 2
18 hours	84 ± 2	77 ± 1*	83 ± 2	95 ± 3	94 ± 1	93 ± 2
27 hours	90 ± 3	82 ± 1*	79 ± 1*	83 ± 1	85 ± 1	93 ± 3*
52 hours	86 ± 2	81 ± 1*	76 ± 1*	73 ± 2	77 ± 2	86 ± 4*

Values represent **mean ± SE** insulin levels in μU/ml; ***,p<0,05** versus control.

Conclusions: TNF-α suppresses, while leptin stimulates insulin secretion *in vitro*. These opposing effects of TNF-α and leptin may be partly mediated via changes in somatostatin expression and might have implications for β-cell function in obese individuals, or in conditions of inflammatory stress or cancer cachexia.

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INHIBITORY EFFECT OF LEPTIN ON INSULIN SECRETION. STUDY IN THE PERFUSED RAT PANCREAS.

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Aims: The effect of leptin on insulin release is a matter of controversy. We have investigated the effect of exogenous leptin on insulin secretion elicited by secretagogues which act on the B-cell via different mechanisms. **Materials and Methods**: The study was performed in the isolated perfused rat pancreas. Perfusate consisted of Krebs-Henseleit buffer supplemented with dextran (4 %), albumin (0.5%) and glucose (5.5 mmol/l). **Results**: Leptin (100 nmol/l) did not affect either basal ($F = 0.73$; n.s.) or the insulin response to increasing glucose concentrations - from 5.5 to 9 mmol/l - (incremental area: 55±5 vs. 47±7 ng/20 min in control experiments; $p=0.6$). However, leptin, at this concentration, markedly inhibited the insulin release elicited by 1 nmol/l CCK-8 (9.2±3 vs. 30±3 ng/10 min in control experiments; $p<0.05$). This octapeptide promotes receptor-linked phosphoinositide hydrolysis, formation of inositol-triphosphate and generation of diacylglycerol which activates B-cell protein kinase C. Leptin (100 nmol/l) significantly reduced the insulin release elicited by 1 μmol/l carbachol (16±5 vs. 42±8 ng/10 min in control experiments; $p<0.05$), a substance which also stimulates insulin secretion affecting phospholipid turnover. **Conclusions**: While leptin does not behave as a general inhibitor of insulin release, it exerts an insulinostatic effect when insulin output is stimulated by secretagogues acting on the B-cell by promoting phospholipid turnover.

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Leptin normalizes islet glucose-6-phosphatase activity and improves insulin secretion in ob/ob mice

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We have previously demonstrated an increased activity of glucose-6-phosphatase in ob/ob mouse islets. In the present study we evaluated the effect of leptin on islet glucose-6-phosphatase activity and insulin release. Seven days treatment with leptin (100 μg/day) to ob/ob mice decreased body weight (6%) and blood glucose levels (48%) compared to vehicles. Islet glucose-6-phosphatase activity was significantly decreased (20.12 ± 2.20 vs 8.79 ± 0.66 pmol/islet/min; $p<0.001$) while glucose stimulated insulin secretion (16.7 mM) was increased (97.09 ± 14.35 vs 176.93 ± 14.47 μU/islet/h; $p<0.001$) in leptin treated mice compared to vehicles. Islet glucose utilization and oxidation as well as triglyceride content (25.33 ± 2.54 vs 36.50 ± 4.26 ng/islet; $p<0.05$) was significantly lower in leptin treated mice compared to vehicles. Addition of leptin in vitro did not influence either insulin secretion or islet glucose-6-phosphatase activity. In conclusion, leptin treatment decreased islet glucose-6-phosphatase activity, triglyceride content and improved insulin secretion in ob/ob mice. We propose that the decreased activity of islet glucose-6-phosphatase contributes to the improvement of insulin responsiveness.

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Glycation

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ADVANCED GLYCATION ENDPRODUCTS (AGEs) IN FOOD STIMULATE THE EXPRESSION OF VCAM-1 IN HUMAN ENDOTHELIAL CELLS.

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Aims: Food AGEs are orally absorbed and metabolized in vivo. But whether these exogenous AGEs activate cellular signalling systems and induce the expression of potentially angiopathic products similar to endogenous AGEs remained unknown. **Materials and Methods:** Therefore, soluble AGE-peptides from instant coffee (Nescafé Gold/Nestlé, Batch WL 3 Z 1955) and from cola-soda (Coca-Cola, Batch LSM 1 9070 J) have been purified by lysozyme affinity chromatography and incubated (150 μg/ml) with primary cultures (n=7) of human umbilical venous endothelial cells (HUVECs) for 6 hrs at 37°C. The stimulation of VCAM-1 expression (ELISA) and NF_kB activation (EMSA) were examined and compared to the analogous effects of purified serum-AGEs from fasting diabetic patients with nephropathy (n=3). **Results:** Compared to controls without AGEs coffee-AGEs stimulated in HUVECs the expression of VCAM-1 by 117%-277% and cola-AGEs by 191%-252%. NF_kB has been activated by these food AGEs in HUVECs to a comparable degree. The purified serum-AGEs stimulated the VCAM-1 expression under the same conditions by 189%-346%. The effect of coffee-AGEs on VCAM-1 expression was not inhibited by RAGE (receptor for AGE) antisense oligonucleotides but was potentiated by RAGE sense oligonucleotides. **Conclusions:** These results support the hypothesis that food AGEs can induce endothelial dysfunction under appropriate circumstances similar to endogenous serum-AGEs and in this way could contribute to the development of micro- and macroangiopathy in diabetic patients.

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ANTIBODIES IN NIDDM SERUM BINDING TO ADVANCED GLYCATION MODIFIED PROTEINS

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Background: The evidence that advanced glycation endproducts (AGEs) have antigenic properties have led to a hypothesis that AGE as an antigen being continuously formed in a diabetic body may arouse an autoimmune response. *The aim* of this study was to investigate the AGE level and anti-AGE response in NIDDM patients (35 without microangiopathy and 15 with nephropathy and/or retinopathy), and in 20 nondiabetic controls. *Methods:* Competitive ELISA was developed to measure AGEs in serum, and inhibition assay was used to detect anti-AGE response. A purified immunoglobulin fraction from human plasma (4500 healthy donors) of normal subclass distribution was used as a standard. AGE-modified IgG was used as antigen to detect anti-IgG-AGE positive samples. *Results*: The binding inhibition assay showed a significant difference between diabetic subjects and nondiabetic controls (36.2±3.9% *vs* 26.4±3.6%, $p<0.0004$) (mean±SE). There also was a significant difference in AGE levels between diabetic patients and control subjects (51.6±4.2 *vs* 19.8±1.7 AU/mg, $p<0.001$). The binding inhibition percentage differed between the diabetics with nephropathy and/or retinopathy (42.0±7.2%), and those free from complications (33.5±4.6%), but the difference was not statistically significant. Spearman correlation showed an inverse relationship between the binding inhibition percentage and parameters of nonenzymatic glycation, HbA1c ($r= -0.4$, $p<0.002$) and AGE level ($r= -0.35$, $p<0.004$). Anti-IgG-AGE positive individuals were detected in both NIDDM groups. *Conclusion:* These data indicate a stronger immune response to proteins modified by advanced glycation in diabetic subjects than in healthy age-matched controls. Long-term presence of both antigens and antigen-specific antibodies might be the cause of a series of undesired phenomena. There is a possibility that antiAGEab form immune complexes which are believed to have a role in atherogenesis. Therefore, additional studies are needed to establish the role and significance of autoantibodies against AGEs in atherogenic processes in diabetes.

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MONOCYTE EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR IS UPREGULATED BY GLYCATED ALBUMIN

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Aims: One hypothesis relating to the pathogenesis of diabetic vascular disease proposes that monocytes bind to advanced glycation end products (AGEs) on the vascular wall and are activated to produce a variety of cytokines which they deliver to the subendothelial space. Of the cytokines which have been suggested as playing a role in diabetic angiopathy, vascular endothelial growth factor (VEGF) has the best case. We therefore aimed to show that monocyte expression of vascular endothelial growth factor could be upregulated by glycated albumin. **Materials and methods:** Monocytes were cultured from peripheral blood of non-diabetic individuals at a concentration of 10^6 cells/ml culture medium. Cells were incubated with glucose (11mmol), bovine serum albumin (1mg.ml^{-1}), human albumin (Sigma, 1mg.ml^{-1}), or glycated albumin (Sigma, 1mg.ml^{-1}) for twenty-four hours. **Results:** Following incubation with glucose, monocytes produced a VEGF mean (SD) concentration of 135 (19.9) pg.ml^{-1}. Monocytes incubated with bovine serum albumin produced a VEGF concentration of 134 (17.8) pg.ml^{-1}. Monocytes incubated with human albumin, with a fructosamine level of 20umol/g protein, produced an increase in VEGF to 188 (130) pg.ml^{-1} (p=0.2). Monocytes incubated with glycated albumin (Sigma) at 1mg.ml^{-1} further increased the VEGF expression to 235 (96) pg.ml^{-1} (p=0.02). **Conclusion:** We conclude that glycated albumin upregulates VEGF expression in human monocytes and supports the hypothesis that activation of monocytes by AGE in diabetic subjects could play a role in the pathogenesis of diabetic angiopathy.

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THE IN VIVO GLYCATION OF IgG: A NEW METHODOLOGICAL APPROACH.

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Non-enzymatic protein glyco-oxidation is a relevant process in diabetes and, in principle, glycated proteins exhibit a different functionality from that of unglycated ones. In this frame the glycation-induced functional change of immunoglobulins is of particular interest. In the present research the glycation levels of IgG were studied by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry applied to samples from 10 poorly-controlled, type 2 diabetic patients (mean age ±SD 67±3 yrs, mean fasting plasma glucose 21.5±5mM/L, mean HbA1c 11.4±2%), 10 well-controlled type 2 diabetic patients (mean age 61±12 yrs, mean fasting plasma glucose 7.8±1mM/L, mean HbA1c 7.4±0.8%) and 10 normal controls (mean age59±9 yrs, mean fasting plasma glucose 5±0.4mM/L, mean HbA1c 5.3±0.6%). This method, by comparing the molecular weight of glycated and native IgG molecules, reveals the number of glucose molecules which have condensed on the protein, which range from 1 to 5 for healthy subjects, from 5 to 9 for well-controlled diabetic patients and from 10 to 25 for poorly-controlled ones. These data may be related to changes in IgG functionality only if linked with the identification of the most favoured glycation sites. This has been obtained by: i) molecular modelling of the whole protein, by calculating the solvent accessible surfaces of the various lysine residues present in the chain, targets for glycation reactions, and iis) digestion of the protein by papaine and the MALDI analysis of the so obtained Fab and Fc fragments. Experimental data and theoretical calculations are in agreement, showing that 24 lysine residues may be reactive against glucose and that glycation is particularly favoured for lysine residues present in the Fab fragment, indicationg that the possible changes in functionality of glycated IgG are related to impaired Fab.

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SIMPLE NONINVASIVE MEASUREMENT OF SKIN AUTOFLUORESCENCE IN DIABETES MELLITUS

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Introduction: Tissue autofluorescence has been associated with presence of advanced glycosylation endproducts (AGE's). AGE assays require tissue sampling or serum Hb-AGE levels which do not reflect AGE accumulation.

Aim: to test an Autofluorescence Reader (AFR), designed to noninvasively measure skin autofluorescence in diabetic patients and matched controls.

Materials and methods: 46 diabetic patients (25 type 1, 21 type 2, age 50±16 yr (mean+SD) were matched for age and gender with 46 controls. After control reflection measurements using a white teflon block. Fluorescence of the skin was measured six times on both lower arm and lower leg (excitation 300-420nm nm, Black light 8 W; emission 420-600nm, Ocean optics PC 1000). As parameters of fluorescence we used total autofluorescence (AFt) which is the summation of all intensities between 420-600 nm, and AFr which is AFt corrected for the amount of light reflected by the skin. AFr is given if skin reflection measurements differed between both groups, and to analyze correlation with age and HbA1c.

Results: Diabetic patients had a duration of diabetes mellitus of 17±10 yr, and a HbA1c of 7.8±1.1%.

	Diabetics (n=46)	Controls (n=46)
AFt of lower arm (a.u.)	1598±703	1184±618 p=0.004
AFt of lower leg (a.u.)	1637±923	1255±847 p=0.04

The amount of light reflected by the skin was not different between both groups (19±7%). AFr correlates with age ($r > 0.52$, $p < 0.001$). AFr of the leg corrected for age correlates with HbA1c ($r > 0.4$, $p < 0.01$).

Conclusions: Using a noninvasive technique, patients with diabetes mellitus have a higher autofluorescence of the skin compared to matched controls. The relation with age and HbA1c strongly suggest that our noninvasive technique offers a viable alternative to measure AGE's.

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Cardiac Complications and Autonomic Neuropathy

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QT DISPERSION INCREASED IN NONDIPPER DIABETIC PATIENTS

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Diabetic patients with autonomic neuropathy (AN) have incresed QT dispersion which is a marker of arrhytmogenic potential and this may explain the increased incidence of sudden death in diabetics with AN. The aim of this study is to evaluate the relation between altered circadian blood pressure pattern, QT dispersion and AN in type 2 diabetics. We performed 24 hour ambulatory blood pressure monitoring; 24 hour urinary albumin excretion measurements, AN tests in healthy controls (C) (n=13), normoalbuminuric (N)(n=31) and microalbuminuric (M)(n=20) type 2 diabetics . Rest 12-lead electrocardiograms were recorded for measurement of QTcd dispersion, defined as the longest QT interval minus the shortest QT interval, corrected for heart rate using Bazett's formula. Although there was no significant difference between day and night blood pressures and heart rates between groups, the amount of dipping of sistolic, **diastolic** blood pressure and MAP in **M** (5.5±3;**9.6±5**;7.1±4 %) was decreased compared with **C** (10±2;**14.9±3**;12.3±2%) (p<0.001;**p<0.003**;p<0.001) and N(10.1±4; 8.3±4 %) (p<0.04). Prevalance of non-dippers was increased in **M**(83%; p<0.0002) and **N**(57%;p<0.01) compared with **C**(18%). Prevalance of **AN** was higher in the non-dipper group(40%) than in the dippers(0%;p<0.003) and **C**(0%;p<0.006). Diabetics as a whole were found to have increased QTc dispersion (QTcd) compared with **C** (71.5 ± 26.0 vs 46.5 ± 13.9 msec; p < 0.01). Patients both with (n: 11) and without (n:40) **AN** had increased QTcd compared with the **C** (85.2 ± 30.8, p < 0.01 and 70.9 ± 22.7, p < 0.05 vs 46.5±13.9 msec). The QTcd of **AN** (+) and **AN** (-) patients were similar, but nondipper diabetics had increased QTcd (75.46±24.6 msec) compared to **C** (46.5±13.9 msec)(p<0.01). QTcd (71.93±26.1) of dipper diabetics was not different from both **C** and nondippers. In conclusion, diurnal rhytm of blood pressure is disturbed even in normoalbuminuric diabetics. Patients with type 2 DM have increased QT dispersion irrespective of the presence of diabetic autonomic neuropathy with the exception of dipper diabetics whose QT dispersion time was not different from healthy controls.

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QT INTERVAL PROLONGATION AND MORTALITY IN TYPE 1 DIABETIC PATIENTS: A 5-YEAR COHORT PROSPECTIVE STUDY.

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The association between QT interval prolongation on resting ECG and excess mortality in diabetic populations has been suggested in non diabetic populations and, recently, in newly diagnosed type 2 diabetic patients. **AIM** : to assess the relationship between corrected QT (QTc) and mortality in type 1 diabetic patients. **METHODS**: We assessed neuropathy and QTc in a random sample of 379 out of all 766 type 1 diabetic patients attending 22 outpatients clinic in Piemonte, Italy and 118 healthy subjects matched for sex and age. This sample was representative of the type 1 diabetic population in the area of the study. Data on survival after 5 years were obtained in 316/379 patients (83.3%) and 106/118 control subjects (90.5%). The patients lost at follow up were not significantly different in age, duration of disease, body mass index, blood pressure and prevalence of autonomic neuropathy. **RESULTS**: Mortality at 5 years was 6.32% among diabetic patients and 0.9% in the control group (p < 0.001). Patients who survived had a significantly different age (31.4±11.1 vs 36.7±10.7 years, p=0.04), duration of diabetes (13.6±8.7 vs 18.7±10.9 years p=0.01), systolic (123.9±20.0 vs 138.0±35.6 mmHg, p=0.004) and diastolic blood pressure (80.5±13.6 vs 83.6±11.2 mmHg, p=0.03) and QTc (0.41±0.03 vs 0.45±0.02 sec, p=0.000005) compared to those who died. At univariate analysis patients had a higher risk of dying (odd risk, 95% CI) if they had a prolonged QTc (20.14, 5.7-70.8) or were affected by autonomic neuropathy (3.55, 1.4-8.9). QTc prolongation was the only variable which showed a significantly higher mortality odd risk at multivariate analysis (p = 0.000004). **CONCLUSION**: This is the first cohort-based prospective study indicating that QTc prolongation is predictive of increased mortality also in type 1 diabetic patients. Even though the mechanism linking QTc prolongation and the excess mortality remains to be elucidated, QTc interval analysis is a simple non-invasive test that could be used to stratify the death risk in diabetic patients, particularly those who are candidates to surgery or kidney and/or pancreas transplantation.

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THE RELATIONSHIP BETWEEN CLINICAL CORONARY EVENTS AND CORONARY ARTERY CALCIUM AS DETECTED BY THE ELECTRON-BEAM ULTRAFAST CT SCAN IN DIABETES.

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Aims: The leading cause of death in diabetes is coronary disease and 25% mortality rates occur at 1 month after a first coronary event and 50% mortality is observed by 1 year. Prevention is thus important, and surrogate testing by noninvasive Electron-Beam Ultrafast CT Scan (EBCT) correlates cross-sectionally with atherosclerosis. We tested the hypothesis that EBCT coronary calcium scores in diabetes can predict future clinical coronary events. **Methods and Materials:** 209 diabetic adults without a clinical history of coronary disease, and of mean age 67 ± 7 years, underwent EBCT and were followed for a mean duration of 40 ± 8 months while longitudinally monitored for coronary events, *e.g.,* coronary death or nonfatal myocardial infarctions, with yearly visits or direct phone contact. Subjects were grouped into tertiles and EBCT calcium scores and event rates were compared using Chi Square analysis. **Results:** Patients comprising the lowest tertile had calcium scores of 0-14 (n=69), and had three events; those in the highest tertile had scores of 238-4575 (n=70), and had five events (p=N.S. vs. lowest tertile); whereas diabetic patients in the mid-tertile having calcium scores of 14-238 (n=70), had nine events (p<.01 vs. lowest tertile). **Conclusion:** These data suggest that 1) more coronary events occur in diabetic patients with mid-level coronary calcium scores when compared with either lower or higher scores, 2) the EBCT calcium score is a useful surrogate for predicting coronary events and 3) in the atherosclerotic process at higher calcium scores, there is more mature, stabilized and calcified plaque when compared to the more unstable, less calcified plaque associated with mid-range scores.

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CARDIAC AUTONOMIC FUNCTION IS ASSOCIATED WITH CORONARY ARTERY CALCIFICATION IN DIABETIC AND NON-DIABETIC PEOPLE

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Aim: To examine the association between cardiac autonomic function and coronary artery calcification (CAC) in diabetic patients and the general population. **Methods**: CAC is highly correlated with the volume of coronary atheroma (r>0.9) and is thus a good measure of coronary artery disease. Using electron beam computed tomography, CAC was quantified in 190 men and 196 women with (51%) and without type 1 diabetes aged 30-50 yrs randomly sampled from diabetic clinics and the general population, respectively. As a measure of cardiac autonomic function, power spectral analysis with autoregressive modeling of resting 5 min tachograms was used to define the total power (TP) within the low and high frequency bands of heart rate variability. TP data were log transformed. **Results:** CAC prevalence was similar in diabetic (52%) and non diabetic (52%) men. In women, CAC was much more prevalent in those with diabetes than without (47% vs 21%, Odds ratio =3.6, 95% CI:2-7, p=<0.0001 adjusted for age). In those with diabetes, TP was strongly associated with CAC (OR=0.68 95% CI:0.5-0.9, p=0.006) independent of age, sex, HbA1c, diabetes duration, albumin excretion rate, triglycerides and HDL and LDL-cholesterol. Adjustment for systolic BP and BMI slightly attenuated the association between TP and CAC so that it was no longer significant (OR=0.7 95% CI 0.5-1.2, p=0.1). TP showed a similar association with CAC in the non diabetic group. Geometric mean TP was significantly lower in those with (358ms^2) than without (733ms^2) diabetes (p<0.001). Adjustment for TP had only a small effect on the diabetes associated odds ratio of CAC in women (reducing it from 3.6 to 3). Further adjustment for SBP reduced this odds ratio to 2.4. **Conclusion:** (i)Cardiac autonomic function is associated with CAC, in people with and without diabetes. This is consistent with autonomic function being disrupted by even subclinical atherosclerosis or autonomic function being aetiologically implicated in atherosclerosis(ii)However cardiac autonomic dysfunction is not a large component of the high risk of CAC in diabetic women.

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PREVALENCE AND CAUSES OF LEFT VENTRICULAR HYPERTROPHY IN NON-ALBUMINURIC TYPE 2 DIABETIC PATIENTS WITHOUT CORONARY HEART DISEASE

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AIMS: To study the prevalence and risk factors for development of left ventricular hypertrophy (LVH) in non-albuminuric type 2 diabetes. **MATERIALS AND METHODS**: M-mode echocardiography was performed by one experienced examiner during 1994-1998 in 348 consecutive, non-albuminuric (urinary albumin excretion rate <100 mg/24h) type 2 diabetic patients with blood pressure <160/95 mmHg. Due to technical difficulties 41 patients could not be evaluated, thus 307 (132 women) patients were included in the study: age(mean(SD)) 55 (10) years, known duration of diabetes 5 (range 1-29) years. Body mass index (BMI) was 28 (5) kg/m^2, HbA_{1c} 8.7 (1.6) %, blood pressure 134 (14) / 80 (8) mmHg, urinary albumin excretion rate (median (range)) 5 (2-89) mg/24h (15% microalbuminuria) and 239 (78%), 64 (21%), 4 (1%) had nil, simplex, proliferative retinopathy, respectively. **RESULTS**: The prevalence of LVH was 31% (95% CI 26-36%). LVH was present in 51 (39%), (CI 31-47%) women and 44 (25%), (CI 19-31%) men, p=0.01. Patients with LVH were older (p=0.01) and had higher: BMI (p=<0.001), HbA_{1c} (p=0.004) and urinary albumin excretion rate (p<0.001) as compared to patients with normal left ventricular mass. There was no significant difference between patients with LVH and patients with normal left ventricular mass regarding systolic and diastolic blood pressure, duration of diabetes or presence of retinopathy. A logistic regression analysis revealed that age, BMI, HbA1c and log urinary albumin excretion were independent risk factors for left ventricular hypertrophy (R^2 adjusted = 0,71). Similar results were obtained for left ventricular mass index. **CONCLUSION**: Our study shows that LVH is frequent in non-albuminuric patients with type 2 diabetes, especially in women. Several potentially modifiable risk factors such as raised BMI, poor glycaemic control and elevated urinary albumin excretion rate predicts the development of LVH.

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Epidemiology of Type 2 Diabetes

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PREVALENCE RATES AND ADDITIONAL FEATURES OF METABOLIC, PLURIMETABOLIC AND INSULIN RESISTANCE SYNDROMES.

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Aims. The present study aimed to establish prevalence rates and additional clinical features of three syndromes which might be identified in the general population. **Materials and Methods.** Within a population-based survey examining 888 subjects aged 40-79 years, subjects were identified fulfilling the following criteria: BMI<25, normal lipids and urate, no hypertension, normal glucose tolerance, normal insulin sensitivity, as assessed by Homeostasis Model Assessment (Controls); normal lipids and urate, no hypertension, normal glucose tolerance, low insulin sensitivity (Insulin Resistance Syndrome, IRS); any association of three disorders among glucose intolerance, dyslipidemia, hypertension and hyperuricemia (Metabolic Syndrome, MS); combination of glucose intolerance, dyslipidemia, hypertension and hyperuricemia (Plurimetabolic Syndrome, PMS). **Results.** The prevalence rates were as follows: IRS 9.6% (95% C.I. 7.7-11.5), MS 6.8% (5.1-8.5), PMS 2.4% (1.4-3.4). The prevalence of PMS, but not that of MS or IRS, was higher in women. The prevalence of PMS and MS was higher in older subjects (age >60) and in overweight-obese subjects (BMI >25), whereas that of IRS was higher in younger individuals and was not different in those with BMI lower or higher than 25. The prevalence of PMS and MS was higher in subjects with low physical activity, whereas the prevalence of IRS was higher in light- or no-drinkers. Subjects with IRS showed modestly but significantly higher levels of glucose, lipids, blood pressure and urate. These subjects had an incidence of metabolic disorders higher than in Controls during a 5-yr follow-up. Subjects with MS and PMS showed higher levels of fibrinogen, apolipoprotein B, FFA, gamma-GT, leukocytes, erythrocytes, leptin and circulating endothelial adhesion molecules (E-selectin and ICAM-1). All these differences remained significant after adjusting for sex, age, BMI, WHR, smoking, alcohol, physical activity and social status. **Conclusions.** An isolated insulin resistance (IRS) and the combination of multiple metabolic disorders (MS and PMS) occur frequently in the general population, and are associated with several non–metabolic abnormalities. Isolated IR predicts the development of metabolic disorders.

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INFLAMMATION AND THE INSULIN RESISTANCE SYNDROME IN HEALTHY SUBJECTS. THE INSULIN RESISTANCE ATHEROSCLEROSIS STUDY (IRAS).

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Aims: Recently, inflammation has been associated with the development of atherosclerosis, and several components of the insulin resistance syndrome (IRS) have been related to inflammatory markers, such as C-reactive protein (CRP). We hypothesized that insulin sensitivity and/or insulinemia may be associated with inflammation and studied the relation of inflammatory markers (CRP, fibrinogen, and white cell count) to components of the IRS.

Methods: Insulin sensitivity (S_I) was measured by a frequently sampled intravenous glucose tolerance test in 1088 non-diabetic subjects free of clinical coronary artery disease (age: 40-69 years). CRP was measured by a highly sensitive competitive immuno-assay.

Results: All 3 inflammatory markers were correlated with several components of the IRS, including fasting insulin and S_I. Strong associations were found between CRP and measures of body fat [BMI (r=0.42), waist circumference (r=0.32)], S_I (r=-0.38), fasting insulin (r=0.34) and proinsulin [intact (r=0.28) and split (r=0.31)] (all p values < 0.0001). The associations were consistent among the 3 ethnic groups of the IRAS population (Blacks, non-Hispanic Whites, Hispanics). There was a linear increase in CRP levels with an increase in the number of metabolic disorders (dyslipidemia, upper body adiposity, insulin resistance, and hypertension). BMI (p=0.0001) and S_I (p=0.003) were related to CRP levels in a multivariate linear regression model after adjusting for age, gender, ethnicity, clinic, and smoking status.

Conclusions: We suggest that chronic subclinical inflammation is part of the insulin resistance syndrome. CRP, a predictor of cardiovascular events in previous reports, was independently related to S_I. These findings suggest potential benefits of anti-inflammatory or insulin-sensitizing treatment strategies in healthy individuals with features of the IRS.

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FACTOR ANALYSIS OF THE VARIABLES INCLUDED IN THE METABOLIC SYNDROME.

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Several previous studies have demonstrated a clustering of hyperinsulinaemia, glucose intolerance, hypertension, dyslipidaemia and (abdominal) obesity often referred to as "the metabolic syndrome" or "the insulin resistance syndrome". It has been proposed that insulin resistance could be the underlying pathophysiological mechanism responsible for the clustering of the variables included in the syndrome. Many previous epidemiological studies have, however, used indirect measurements for insulin resistance in the analyses. **Aim:** The aim of this study was to apply factor analysis to the EGIR data in order to identify the associations between the variables, and the factors (i.e. etiological processes) underlying the clustering of the variables. **Materials and Methods:** The EGIR database contains data from the euglycaemic hyperinsulinaemic clamp studies in normal glucose tolerant subjects performed at 20 European centers, a technique which is considered to be the "golden standard" measure for insulin resistance. We included 364 subjects in the analyses (207 men; 157 women). **Results:** We identified 3 factors underlying the clustering of the variables included in the syndrome. The first factor was an association with body mass index (BMI), fasting plasma insulin, insulin sensitivity, HDL-cholesterol and triglycerides. Fasting plasma glucose, total-cholesterol and triglycerides were associated with a second factor. Systolic and diastolic blood pressure were associated with a third factor. The results indicate a role for three independent patophysiological processes underlying the clustering - 1) a core complex of abnormalities including insulin sensitivity, dyslipidemia and obesity, 2) glucose levels, and 3) blood pressure. Fasting glucose levels are peripherally linked to this core complex through triglycerides, whereas factor 3 including systolic and diastolic blood pressure share no components with the other two factors. **Conclusions:** The findings do support the notion of insulin resistance, abdominal obesity and dyslipidemia as common denominators for the development of the metabolic syndrome, but questions the role of hypertension and glucose intolerance as core components.

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HAEMATOCRIT AND MEASURES OF OBESITY. THE HOORN STUDY

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Recently, it has been shown that haematocrit is associated with insulin resistance measured with a euglycemic clamp. This is in line with prior studies that found associations of haematocrit with body mass index (BMI). A possible association of waist-to-hip ratio (WHR) with haematocrit has not yet been investigated in a large population study. **Aims:** To study whether haematocrit is associated in a general population with WHR and/or BMI. **Materials and Methods**: Using the data of the Hoorn study, a study of glucose tolerance among 1141 men and 1324 women, aged 50-75 years, we investigated by means of multiple linear regression analyses, the age-adjusted association of haematocrit with WHR and with BMI in men and women. **Results:** There were significant associations of BMI with haematocrit in men ($\beta = 0.08$ % per kg/m^2, $P = 0.02$) and women ($\beta = 0.08$ % kg/m^2, $P < 0.001$). The association of haematocrit with WHR was also significant in men ($\beta = 5.5$ % per m/m, $P < 0.001$) and women ($\beta = 8.6$ % per m/m, $P < 0.001$). This slope was significantly steeper in women than in men ($P = 0.02$). Adjustment of the associations of WHR and BMI with haematocrit for each other caused the associations with BMI to disappear in men and women, and with WHR to remain unchanged in both genders. **Conclusions:** The stronger association of WHR with haematocrit than BMI is an argument in favor of haematocrit as component of the insulin resistance syndrome. The steeper slope of the association of WHR with haematocrit in women may be explained by the known positive association of serum androgens, which stimulate the erythropoiesis, with WHR in women.

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WEIGHT GAIN, β-CELL SECRETORY ACTIVITY AND INSULIN SENSITIVITY IN SEVEN YEAR OLD CHILDREN.

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Background: Studies have shown that NIDDM is more prevalent in individuals of low birth weight who are obese as adults. There is a high prevalence of small for gestational age births and obesity in South Africa and this implies that the incidence of Type 2 diabetes in this country will rise. **Aim**: To study the relationship between birth weight, post natal weight gain and glucose tolerance in a cohort of 7-year-old South African children taken from an on-going longitudinal study of childhood growth (the Birth to Ten Study).

Materials and Methods: Oral Glucose Tolerance tests were carried out on 152 children according to W.H.O. criteria. Blood samples were drawn at 0,30 and 120 minutes. Glucose, insulin, proinsulin, des-31-32 proinsulin and non esterified fatty acids (NEFA) were measured. Children born below median birth weight were divided into two groups: those who at age 7 remained below the median for weight (Group 1) and those who had a weight above the median value (Group 2). **Results**: Group 2 children had significantly higher BMIs ($P<0.01$). they also had lower NEFA levels but higher 120' insulin ($p<0.05$), fasting ($p<0.05$) and 30' ($p<0.01$) des-31-32 proinsulin and 120' ($p<0.0005$) proinsulin levels. Glucose levels were similar in the two groups. **Conclusion**: Children at age 7 who had gained more weight than children born with similarly low birth weights display signs of the metabolic and morphological characteristics of type 2 diabetes i.e. reduced sensitivity to insulin in terms of glucose uptake, high proinsulin-related peptide levels and increased adiposity. However, these children are more sensitive to insulin inhibition of lipolysis and we hypothesize that this maybe a post natal adaptation to high nutrient intake.

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LACK OF EFFECT OF ONE-YEAR ORAL INSULIN THERAPY IN RECENT ONSET TYPE 1 DIABETES: results of a multicenter randomised controlled trial.
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Aims: Oral administrations of antigens can slow down the pathogenetic process in autoimmune diseases, including Type 1 diabetes (IDDM) in the NOD mouse. The aim of the present study was to assess whether continuous oral administration of human biogenetic insulin (2.5 or 7.5 mg/day over a one-year period) could preserve endogenous insulin secretion in autoantibody-positive (ICA and/or GADA) patients with newly-diagnosed (≤ 15 days) IDDM. **Patients and Methods**: From 1996 to 1998, 163 patients aged 7 to 40 years (71 less than 15 years), without severe ketoacidosis at diagnosis (pH ≥ 7.20, weight loss ≤ 10%, polyuria ≤ 6 weeks), have been randomly assigned to daily doses of 2.5 or 7.5 mg oral insulin (Lilly), or placebo, in addition to optimized subcutaneous insulin treatment. Fasting, glucagon- and mixed meal-stimulated C peptide (CP) levels, as well as HbA1c, parenteral insulin requirements and humoral immunological markers, were assessed every 3 months. **Results**: HbA1c and CP levels were similar in tne 3 groups at entry in the study. At 6 and 12 months, subcutaneous insulin requirements and HbA1c levels were not influenced by either dose of oral insulin. Similarly, fasting, glucagon- and meal-stimulated CP values were not significantly different between placebo or insulin-treated groups at 6 and 12 months. Mean CP values at 12 months were: fasting CP: 0.17, 0.14 and 0.15 nM; glucagon-stimulated CP: 0.33, 0.33 and 0.34 nM; meal-stimulated CP: 0.58, 0.61 and 0.48 nM for placebo, 2.5 mg and 7.5 mg oral insulin groups, respectively. The lack of effect of oral insulin was observed both in patients aged less than 15 years as well as more than 15 years. Detailed analysis, including the course of humoral immunological markers will be reported. **Conclusions**: These results suggest that oral insulin, at least at doses used in the present trial, do not slow down the deterioration of ß-cell function during the first year after diagnosis of type 1 diabetes.

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USE OF ISLET CELL ANTIBODY ASSAY TO IDENTIFY TYPE 1 DIABETIC PATIENTS WITH RAPID DECREASE IN C-PEPTIDE LEVELS FOLLOWING CLINICAL ONSET.
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Aims: A rapid decrease in β cell mass and C-peptide levels has been noted in some, but not all, recent-onset type 1 diabetic patients. The present study investigated whether antibody markers could help predict such decline at diagnosis. **Methods:** We measured random C-peptide levels (RIA) and antibodies against islet cell cytoplasm (ICA; indirect immunofluorescence), 65 kDa glutamate decarboxylase (GADA) and IA-2 protein (IA-2-A; liquid phase radiobinding assays) in 172 insulin-requiring type 1 diabetic patients under age 40 years consecutively recruited at diagnosis by the Belgian Diabetes Registry and followed for 2 years. **Results:** Two years after diagnosis random C-peptide levels had significantly ($p<0.001$) decreased in ICA-positive patients but not in ICA-negative patients. This decrease occurred regardless of the presence or absence of IA-2-A or GADA. C-Peptide values below 0.15 µg/L were noted in 88% of patients diagnosed before age 7 years, in 45% diagnosed between 7 and 15 years and in 29% diagnosed thereafter ($p<0.001$). In case of clinical onset before age 15 years, a rapid decline in random C-peptide values was almost exclusively observed in patients with high titer ICA (≥ 50 JDF units) at diagnosis (34 of 49 or 69% vs 3 of 18 or 17% in patients with ICA titer < 50 JDF units; $p<0.001$). ICA prevalence and titers were overall lower in patients diagnosed after age 15 years. In this age group, 25 of 70 patients with ICA titer ≥ 12 JDF units (36%) developed low C-peptide levels compared to 5 of 35 (14%) with ICA titer < 12 JDF units ($p<0.03$). **Conclusions:** Young age at diagnosis and presence of ICA, but not GADA or IA-2-A, identify a group of type 1 diabetic patients at high risk of rapidly losing residual β cell function. Beta-cell preserving strategies should primarily be tested in this subgroup. The ICA assay measures clinically relevant antibodies with specificities or affinities that are not detected in the molecular antibody assays using recombinant human islet cell autoantigens for substrate.

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ß-CASEIN A1 MIGHT BE A POTENTIAL ANTIGEN IN TYPE-1-DIABETES MELLITUS
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Aims: Various components of cow's milk have been investigated as a possible environmental factor in the pathogenesis of type I diabetes mellitus (IDDM). Studies in animal models show that NOD mice do not develop IDDM when fed on a casein-free diet. There have been no major studies thus far on the milk protein ß-casein A1 and A2 to evaluate etiology, prevalence and pathogenesis of IDDM. In this clinical family study, for the first time, antibodies against the most common genetic variants of ß-casein have been detected in such a large sample. **Materials and Methods:** Altogether, sera of 1257 persons (287 type 1 diabetics, 386 siblings, 477 parents and 107 healthy controls) were studied for ß-casein A1- and A2-antibodies. Investigations were carried out by enzyme-linked immunosorbent assay (ELISA). **Results:** Antibodies to both genetic variants of ß-casein were found most frequently in diabetics. For all four groups high titers of casein A1 and A2 antibodies were strictly correlated with low age which was statistically significant ($p<0.001$). A qualitative comparison of ß-casein A1 and A2 antibodies showed more casein A1 antibodies in diabetics and their siblings whereas in parents and controls more casein A2 antibodies were found. The preferential binding of the sera to the one or the other variant of ß-casein was statistically significant ($p<0.001$) in all four groups. **Conclusion:** In summary, the A1 variant of ß-casein might be associated with IDDM. On the other hand, the A2-variant might more likely play a protective role. We could show that ß-casein A1 could be a possible potential antigen in the pathogenesis of diabetes mellitus. Further investigations are necessary to confirm the role of ß-casein A1 in the pathogenesis of IDDM.

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THE INNATE ANTIVIRAL DEFENSE SYSTEM IS PERSISTENTLY ACTIVATED IN HUMAN TYPE 1 DIABETES
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Type 1 diabetes (IDDM) results from autoimmune destruction of the pancreatic α-cells. The factors initiating this destruction have not yet been identified although viruses have been implicated. Recently, attention has been increasingly focused on the role of the innate antiviral defense system in directing adaptive immune responses. In this context, the pathogenesis of IDDM may have an aberrant response to endogenous or exogenous viruses or their products. Hence the **aim** was to examine the antiviral defense system expressed as the IFN-α inducible 2′,5′ oligoadenylate synthetases (2′,5′AS) which are ds/ss RNA-dependent isoenzymes. **Methods**: Activities of 2′,5′AS and proteinkinase p68 were determined enzymatically in lymphocytes homogenates by ^{32}P-labelled ATP and quantified by β-counting or phosphorimaging. The **results** show that lymphocytic 2′,5′AS activity is significantly and selectively increased in type 1 diabetics, both in those with recent-onset (n14, $p<0.005$) and with long-standing disease (n=24, $p<0.001$) as compared to healthy controls (n=65). Activity of 2′,5′AS was not elevated in patients with type 2 diabetes or multiple sclerosis, thus excluding hyperglycemia or autoimmunity *per se* as inducing upregulation of enzyme activity. Surprisingly, lymphocyte levels of PKR proteinkinase and MxA, two other IFN-α inducible antiviral proteins, were similar in IDDM and controls. This suggests that the increased 2′,5′AS activity is not due to a general induction of IFN-α pathways, but rather is an aberrant response to viruses or RNA molecules, originating from exogenous or endogenous sources.

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TH1-LIKE DOMINANCE IN HIGH RISK FIRST-DEGREE RELATIVES OF TYPE 1 DIABETIC PATIENTS

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Background The humoral part of the immune system including autoantibodies is known to predict mainfest type 1 diabetes in first degree relatives while the cellmediated immune process preceding the manifest disease is still unknown.

Aim To estimate the immunological balance of Th-like lymphocytes (Th1/Th2) in high-risk first-degree relatives of type 1 diabetic children.

Patients and methods Human peripheral blood mononuclear cells (PBMC) from twenty-one healthy first degree relatives (7-48 yrs) with high risk of developing type 1 diabetes (ICA ≥20) were examined and compared with the response seen in PBMC from six children with newly diagnosed type 1 diabetes as well as from six healthy controls with similar age, sex and HLA-type. IL-4 and IFN-γ were determined at the level of transcription as mRNA by RT-PCR and as protein by ELIspot after stimulation with specific epitopes of Glutamic Acid Decarboxylase (a.a. 247-279, 509-528, 524-543), insulin, the ABBOS-peptide (a.a. 152-169) as well as Bovine Serum Albumin.

Results Individuals with high risk of developing type 1 diabetes had a high spontaneous secretion of IFN-γ compared to both diabetic children and healthy controls (p=0.0004), lower amounts of IL-4 compared to healthy controls (p=0.07), and a significantly elevated ratio of IFN-γ/IL-4 compared to both diabetic children and healthy controls (p=0.0001). Production of IFN-γ seen in high-risk individuals was negatively correlated to production of GAD_{65} autoantibodies (*rho*= -0.444, p=0.053). The amount of IFN-γ, which was extremely high in high-risk individuals, did not further increase after stimulation with specific peptides, even though the peptide of GAD_{65} (a.a.247-279) which has been associated to Coxsackie B virus caused increased mRNA expression for IFN-γ in high-risk individuals (p=0.086). Increased expression of IFN-γ mRNA caused by this specific peptide of GAD_{65} was related to low production of autoantibodies to GAD_{65} (p=0.1).

Conclusion Overwhelming production of IFN-γ seen in PBMC from high-risk first-degree relatives of children with type 1 diabetes suggests a Th1-like immune deviation in the pre-diabetic phase.

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DO MOBILE CELLULAR PHONES INDUCE MALFUNCTIONING OF IMPLANTED INSULIN PUMPS ?

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Electromagnetic fields radiated by cellular phones affect medical devices such as pacemakers and some models of portable insulin pumps. In a collaborative work with the CNET (Research and development center of (France Telecom), we verified whether radiofrequency emission from mobile cellular phones cause malfunctioning of implantable insulin pumps. We tested in vitro 2 implantable insulin pumps of the same model Minimed MIP 2001 (pumps A and B) with 2 types of cellular phones (DCS Flare B 300 (1800 Mhz, 1 W) with pump A and GSM Lisa P9026 (900 Mhz, 2 W) with pump B). Phones held just over the pumps, were used 1) in test mode 2) with maximal power transmission 3) in actual transmission to simulate actual use. Because volumes delivered by implantable pumps were very small, a special protocol was designed to obtain sufficient amount of insulin: pumps were programmed to deliver maximal basal rate and maximal boluses and were filled with Actrapid HMGE U40 (Novo Nordisk) instead of insulin usually used (21 PH Hoechst U400). Insulin delivered by the pumps was collected in 1 ml buffer and a particular enzyme immunoassay was designed to be sensible enough to detect 2 µU/ml (intraassay CV : 3.2 %, interassay CV : 4.8 %). No alarm activation or change in programmed parameters of the implantable device was observed when cellular phones were used in a test mode or with a maximal power transmission. During simulating tests, the amount of insulin delivered by the pump was measured twice under basal conditions and then twice while cellular phone was used in usual transmission. For pump A tested with phone DCS : bolus were measured at 5.3 and 5.4 U (1st and 2nd measures under basal conditions) vs 5 and 5.4 U (1st and 2nd measures during simulating tests). Basal rate was 0.9 and 0.86 U/h versus 0.86 and 0.85 U/h. For pump B tested with phone GSM: bolus were 5.26 and 4.96 U vs 5.33 and 5.06 U. Basal rate was 0.84 and 0.84 U/h vs 0.86 and 0.86 U/h. So whatever the type of cellular phone calling in contact with the pump, we did not observe any statistically significant modification of the amount of insulin delivered during boluses and basal rate. Both types of cellular phones (1 W or 2 W) when used in close contact with the implantable pump Minimed MIP 2001 do not disturb programmation or functioning of this medical device.

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Breaking the Age Barrier: A Comparison of Continuous Subcutaneous Insulin Infusion in Adolescents and Adults.

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Aims: The utility of continuous subcutaneous insulin infusion (CSII) as a method of intensive insulin therapy has already been demonstrated in adults. CSII use among adolescents has increased more slowly than in adults. The clinical outcomes before and after pump initiation were compared for adolescent and adult patients of a large diabetes practice to confirm the utility of CSII therapy for adolescent patients.

Methods: A total of 272 patients initiated on pump therapy between May 1985 and December 1997 were studied. Forty-three of these patients (16%) were between 11 and 20 years of age when they began pump therapy, and 229 (84%) began pump therapy as adults. HbA1c, total daily insulin dose (TDD), severe hypoglycemia, and diabetic ketoacidosis (DKA) were measured at each office visit.

Results: After 18 months of CSII therapy, both adolescent and adult patients showed a significant decrease in HbA1c values, TDD, and severe hypoglycemia; adults showed a decrease in DKA. When compared to adults, adolescents had significantly higher HbA1c, higher TDD, and higher DKA rates both before and after pump initiation.

Outcome	Adolescents		Adults	
	Pre-Pump	Post-Pump	Pre-Pump	Post-Pump
HbA1c (%)	9.3 ± 2.0*	8.2 ± 1.4[a]	7.9 ± 1.5[c]	7.2 ± 1.2[a,c]
Insulin (Units/day)	65 ± 25	56 ± 18[a]	44 ± 15[c]	37 ± 11[a,c]
Hypoglycemia (Events/yr)	1.0 ± 1.6	0.2 ± 0.5[b]	1.7 ± 3.6	0.3 ± 0.8[a]
DKA (Events/yr)	0.3 ± 0.8	0.1 ± 0.4	0.1 ± 0.6[d]	0.01 ± 0.2[b,d]

**Mean ± SD; [a]p<0.001 & [b]p<0.01 vs pre-pump; [c]p<0.001 & [d]p<0.05 vs adolescents*

Conclusions: These results confirm that adolescents demonstrate an improvement in glycemic control, reduction in insulin dosage, and a reduction in severe hypoglycemia with CSII at least comparable to those experienced by adults.

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USE OF A TELEMEDICINE DEVICE FOR THE CARE OF DIABETIC PATIENTS

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Telemedicine is the use of telecommunications technologies to provide medical information and services. **Aims**: To describe a new telemedicine service for the care of diabetics. **Materials and Methods**: We have developed a hand-held device integrated by a home blood glucose meter (Glucometer Elite, KDK, Japan), an adapted palmtop computer (Psion PLC, UK), and a digital mobile GSM (Ericsson, Sweden). The device communicates with a Central Communication Unit (CCU) through the Short Messages System (SMS) of GSM mobile communications. The CCU communicates with physicians via Internet. In the CCU, computerised clinical records are continuously updated. The result of every blood glucose monitored by the patient is transmitted in real time to the CCU. If that result is within pre-established limits, the CCU answers with a reassurance message. If it is outside those limits, the result (together with previous blood glucose values and a summary of the medical record) is sent via Internet to the patient's physician (with an alert via SMS-GSM). The physician may then answer with the appropriate therapeutic advice. If the physician does not respond, other physician or the CCU physician may take the relay. **Results**: A total of 101 insulin-treated diabetics (aged 17-78 years) have used this system. In 66 patients, the duration of use was short (less than 2 weeks) in order to cover a trip abroad or a metabolic decompensation. In 35 patients, the duration of use was prolonged (3-30 months) due to: isolation/difficult access to medical facilities, inadequate control, life-style, intercurrent disease, pregnancy, etc. In these patients, HbA1c decreased from 7.4% (range: 6.2-11.0%) to 6.4% (range: 4.4-8.9%), $p<0.01$. Episodes of serious metabolic decompensation were reduced to one episode (blood glucose >500 mg/dL) which occurred in a young girl staying abroad in whom the situation could be resolved at home. Two pregnancies have been satisfactory followed. The system was considered highly acceptable by 88% of the patients. **Conclusions**: Telemedicine services, as the one here presented, represent interesting and useful complements for the care of diabetic patients.

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Glucose Monitoring in the Interstitial Fluid Combining Open Flow Microperfusion and Ionic Reference Technique

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Aims: The objective of this study was to investigate the ionic reference technique for glucose monitoring in the subcutaneous (s.c.) adipose tissue of subjects with IDDM using open flow microperfusion (OFM). **Methods:** OFM is based on a double lumen catheter with macroscopic perforations on the outer surface. The catheter was inserted into the s.c. adipose tissue and perfused with mannitol at 0.5 µl/min. The ratio of equilibration (recovery) between the perfusate and the interstitial fluid (ISF) was determined by simultaneous measurement of glucose and ions (ionic reference technique). Seven patients with IDDM (3 men and 4 women) participated in this study. The patients arrived in the morning in a non-fasting state. The double lumen catheter was inserted into the s.c. adipose tissue and every 30 minutes ISF- and plasma samples were collected. During the investigation the patients got a standardised meal and followed their usual pattern of insulin injection. **Results:** The mean recovery determined with the ionic reference technique was 33.4 ± 13.5 % (range: 14.4 – 51.4 %). The estimated ISF glucose concentration paralleled excellently but was lower than the corresponding plasma values (65 ± 4.8 %). The coefficient of variation (CV) of the estimated glucose concentration was significantly lower than the CV of the glucose in the ISF samples (27.7 vs. 8.8 %, $p<0.001$). **Conclusion:** The recovery depends on the individual and can be determined with the ionic reference technique. Furthermore, variations can be reduced or cancelled with this method which enables a more stable and reliable measurement.

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NON-INVASIVE CONTINUOUS GLUCOSE MONITORING DURING ORAL GLUCOSE TOLERANCE TESTS IN VOLUNTEERS AND DIABETIC PATIENTS

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Aims: Continuous glucose monitoring by means of optical glucose sensors would allow diabetic patients to check their metabolic control at their convenience. In experimental glucose clamp studies with patients with type 1 diabetes mellitus we have demonstrated that non-invasive glucose monitoring is possible by registration of the scattering coefficient of human skin. The aim of this study was to evaluate if physiologically slow changes in glycemia can also be monitored. **Material and Methods:** Six healthy volunteers (age 30±10 y, BMI 24.2±5.0 kg/m²) and 13 patients with type 2 diabetes mellitus (age 57±8 y, BMI 29.2±2.0 kg/m²) participated in oral glucose tolerance tests (75 g). With each volunteer/patient two simultaneous measurements were carried out. For registration of the skin tissue scattering coefficient a portable system was used. An optic sensor head was attached directly to the skin and light at different wavelengths was applied for registration of reflected light intensity. In the patients parallel measurements of the interstitial fluid glucose concentration were carried out by means of the microdialysis technique (CMA catheter and analyzer). **Results:** Blood glucose increased from baseline levels of 5.5±0.4 mmol/l to maximal values of 8.2±3.4 mmol/l in healthy subjects and from 8.8±0.8 to 17.1±2.2 mmol/l after 114±17 min in diabetic patients. In 8 of the 12 measurements with the volunteers the observed changes in scattering coefficient correlated well or acceptable with the changes in glycemia (linear regression coefficient r=0.75). A moderate or none correlation was observed in 4 measurements. In 4 volunteers both simultaneous measurements gave comparable results. In 16 of the 26 measurements with diabetic patients a good correlation was found (r=0.77), only a moderate correlation was seen in 2 and no correlation in 9 measurements. In 11 patients both measurements gave comparable results. In the experiments with a good correlation the increase and decrease in glycemia can be monitored. The interstitial glucose concentration showed a good correlation with the intravasal measurements in the patients (r=0.84). **Conclusions:** This study showed that slow changes in blood glucose can be monitored by registration of scattering coefficient changes in volunteers and diabetic patients. It remains to be elucidated why this is not possible in all experiments.

OP 40
Ionic Regulation of Insulin Secretion

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IONIC DEFECTS IN THE CONTROL OF INSULIN RELEASE FROM HUMAN NIDDM ß-CELLS *IN VITRO*.

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Aims: Although the role of ion channels in the control of insulin release from ß-cells is well established, there is little data on their functionality in cells or islets isolated from diabetic donor tissue. Here, we examined stimulus-secretion coupling events in intact islets and in single cells isolated from a diet-treated NIDDM patient who suffered a fatal myocardial infarction. **Methods:** Ion channel regulation was studied in single cells and isolated patches by patch clamp techniques. Intracellular calcium changes ($[Ca^{2+}]_i$) were monitored by fura-2 microfluorimetry, and insulin secretion from single perifused islets was measured using a highly sensitive ELISA. **Results:** Single ion channel recordings from NIDDM ß-cells revealed that only 27% of islet cells possessed functional ATP-sensitive K^+ (K_{ATP}) channels (n=3/11) compared to 100% of recordings from control human ß-cells (n=164). Furthermore, in those cells that possessed K_{ATP} channels, there were no effects of intracellular nucleotides (n=3/3) or tolbutamide (n=3/3) on channel activity. Using the whole-cell configuration, we found that only 50% of cells possessed functional voltage-dependent Ca^{2+} channels (VDCC; n=5/10), compared to 100% of recordings from control cells (n=67). On average, VDCC currents in NIDDM ß-cells were ~50% smaller (Students t-test $p<0.05$) than parallel recordings of VDCC currents in control cells: mean peak current densities were -6.6±1.1 pA/pF (n=5) *vs* -12.9± 1.3pA/pF (n=22), respectively. As a consequence of the ion channel defects, insulin secretagogues that are depolarisation-dependent rarely initiated increases in $[Ca^{2+}]_i$ (20mM glucose n=0/6; 40mM KCl n=3/5; tolbutamide n=1/5), yet cytosolic Ca^{2+} levels were readily elevated by ACh and ATP (100µM each; n=25/26). Finally, secretion studies indicated that the average pulse mass in the diabetic tissue was decreased by around 70% of that measured in control islets. **Conclusions:** These data document a causal relationship between loss of ion channel function in type 2 diabetic β-cells and the disruption of both cytosolic Ca^{2+} signalling events and regulated insulin release.

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STOICHIOMETRY OF SULFONYLUREA ACTION

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Aims: ATP-sensitive potassium channels (K_{ATP} channels) are assembled with an octameric stoichiometry, $(SUR/Kir6.x)_4$, from two structurally distinct subunits, an inwardly-rectifying potassium channel subunit ($K_{IR}6.1$ or $K_{IR}6.2$) forming the pore and a regulatory subunit, a sulfonylurea receptor (SUR), belonging to the ATP-binding cassette (ABC) superfamily. $SUR1/K_{IR}6.2$ reconstitute the neuronal / pancreatic β–cell channel, while $SUR2B/K_{IR}6.1$ (or $K_{IR}6.2$) have been suggested to reconstitute vascular smooth muscle channels. Hypoglycemic sulfonylureas exert their effects on pancreatic β–cells and vascular and non-vascular smooth muscle by interaction with the sulfonylurea receptor subunit thereby closing the channels, depolarizing the membrane potential and increasing cellular electrical activity. However, the stoichiometry of K_{ATP} channel inhibition induced by sulfonylureas was not yet clear and thus we have tackled this problem by comparing the binding affinities of these drugs with their potencies to inhibit reconstituted channels. **Methods:** Following coexpression of SUR1 or SUR2B with $K_{IR}6.2$ in COS-7 cells, channel activity of recombinant channels was measured using the inside-out configuration of the patch-clamp technique. Specific binding of sulfonylureas was assessed by use of [^{3}H]glibenclamide or [^{3}H]P1075 displacement assays. **Results:** Potencies of glibenclamide, glipizide, tolbutamide and meglitinide to inhibit activity of $SUR1/K_{IR}6.2$ and $SUR2B/K_{IR}6.2$ channels were 3-6 fold higher than binding affinities of these drugs with concentration-inhibition relations being significantly steeper (Hill coefficients 1.23-1.32) than binding curves (Hill coefficients 0.93–1.06). **Conclusion: Occupation of one of the four sulfonylurea receptor sites per channel complex is sufficient to induce K_{ATP} channel closure.**

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ROLE OF THE C-TERMINUS OF SULFONYLUREA RECEPTORS IN SULFONYLUREA BINDING

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Aims: ATP-sensitive potassium channels (K_{ATP} channels) are assembled with an octameric stoichiometry, $(SUR/Kir6.x)_4$, from two structurally distinct subunits, an inwardly-rectifying potassium channel subunit ($K_{IR}6.x$) forming the pore and a regulatory subunit, a sulfonylurea receptor (SUR), belonging to the ATP-binding cassette (ABC) superfamily. Three isoforms of SURs have been cloned, a high affinity sulfonylurea receptor, SUR1, and two low affinity receptors, SUR2A and SUR2B. $SUR1/K_{IR}6.2$ have been proposed to reconstitute the neuronal/pancreatic β–cell, $SUR2A/K_{IR}6.2$ the cardiac and $SUR2B/K_{IR}6.1$ (or $K_{IR}6.2$) the vascular smooth muscle-type K_{ATP} channels. SUR2A and SUR2B are splice products of a single gene, differing only in their C-terminal 42-45 amino acids which have been shown to be critical for binding of potassium channel openers (KCOs). In this study we aimed to analyze the role of the C-terminus in sulfonylurea binding. **Methods:** Using DNA-recombination techniques we constructed a chimeric protein, in which the C-terminal 42 aminoacids of SUR2A were replaced by those of SUR1 (SUR2/ct1). SUR2A, SUR2B and SUR1/ct1 were transiently expressed in COS-7 cells and membranes were prepared from these cells. Affinites were measured by [^{3}H]P1075 displacement studies. **Results:** We assessed the affinities of glibenclamide, meglitinide, glipizide and tolbutamide for SUR2A (0.29 µM; 5.3 µM; 6.9 µM or 0.23 mM), SUR2B (0.23 µM; 6.2 µM; 6.0 µM or 0.24 mM) and SUR2/ct1 (0.23 µM; 4.4 µM; 7.2 µM or 0.25 mM). The dissociation constants for these three isoforms were found not to differ significantly. **Conclusion: The C-terminus of SURs – in contrast to its role for KCOs - does not affect sulfonylurea binding and thus most probably does not form part of the binding pocket. The results support the conclusion that sulfonylureas and KCOs exert their effects on K_{ATP} channels via interaction with distinct sites.**

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IMAGING $[Ca^{2+}]$ IN THE IMMEDIATE VICINITY OF SECRETORY VESICLES WITH TARGETED CAMELEONS

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Aim. To image Ca^{2+} concentration ($[Ca^{2+}]$) changes in the immediate vicinity of individual insulin secretory granules in single living islet β-cells. **Materials and Methods.** We constructed cDNA encoding a chimaeric fusion protein between the granule protein phogrin (*pho*sphatase on the *gr*anule of *in*sulinoma), and a fluorescent "cameleon", Ycam-2 - a member of the new family of Ca^{2+}-indicators which rely on fluorescence resonance energy transfer (FRET) between two mutants of green fluorescent protein (GFP). **Results.** After microinjection of cDNA and expression of the chimeara in MIN6 β-cells, monitoring by digital imaging microscopy of the fluorescence intensity ratio (535/480 nm) revealed: (1) larger $[Ca^{2+}]$ increases for granules docked beneath the plasma membrane than those deeper within the cell; (2) heterogeneity in the $[Ca^{2+}]$ changes experienced by individual docked granules after depolarization-induced Ca^{2+} influx. **Conclusions.** Glucose-induced influx of Ca^{2+} through voltage-gated Ca^{2+} channels may lead to spatially confined microdomains of high $[Ca^{2+}]$ beneath the plasma membrane. This may be critical for the triggered exocytosis of insulin from docked granules. Loss of such $[Ca^{2+}]$ microdomains may contribute to defective insulin release in non insulin-dependent diabetes mellitus.

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REGULATION OF ORGANELLE FREE Ca^{2+} IN INDIVIDUAL PANCREATIC β-CELLS BY GLUCOSE, ATP AND AMBIENT Ca^{2+}

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Aims: The regulation of organelle free Ca^{2+} was studied in individual mouse pancreatic β-cells. **Materials and Methods:** The β-cells were loaded with the low-affinity indicator furaptra and studied with dual wavelength microfluorometry. Controlled permeabilization of the plasma membrane with digitonin unmasked indicator remaining trapped within cellular organelles. **Results:** In the presence of ATP there was a rapid accumulation of Ca^{2+} in organelles, the concentration reaching almost 500 μmol/l. At 50 nmol/l Ca^{2+}, which is close to the resting cytoplasmic concentration, the organelle stores became half-maximally filled at 45 μmol/l and saturated at 1 mmol/l ATP with no further effect at 3 mmol/l ATP when gradually increasing Ca^{2+} to 5 μmol/l. At 200 nmol/l Ca^{2+} only 3.5 μmol/l ATP was required for half-maximal filling. In intact β-cells, maintained at close to resting concentrations of cytoplasmic Ca^{2+}, glucose effectively stimulated organelle sequestration of Ca^{2+} with half-maximal and maximal filling at 5.5 and >20 mmol/l, respectively. Since physiological concentrations of cytoplasmic ATP are in the mmol/l range, it can be anticipated that Ca^{2+} sequestration is modulated by a factor reducing the ATP-sensitivity of the transport process. Although phosphatidylinositol 4,5-bisphosphate (PIP_2) was found to mobilize some of the incorporated Ca^{2+} by another mechanism than inositol 1,4,5-trisphosphate, there were no evidence that PIP_2 altered the ATP sensitivity of Ca^{2+} sequestration. **Conclusions:** The data indicate that Ca^{2+} sequestration in β-cells is regulated by a complex interplay between cytoplasmic Ca^{2+}, ATP and factor(s) modulating the ATP sensitivity.

OP 41
Insulin Signalling

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PTP-3 IS A PUTATIVE TYROSINE PHOSPAHATASE REGULATING INSULIN SIGNALING

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Aim: Transfection of CHO cells with the human insulin receptor (CHO/HIRc) not only induces high level of receptor autophosphyralation but it is also characterized by a compensatory rapid receptor dephosphorylation. We utilized this in vitro model to identify the insulin receptor tyrosine phosphatase. **Material and Methods:** Differential display RT-PCR was employed to identify the specific phosphatase. RT-PCR for β-actin was performed to control for non-specific amplification. Northern and Southern blot experiments were performed for gene expression studies. **Results:** Using a differential display strategy we cloned a novel 503 bp DNA fragment (termed PTP-3) largely expressed in CHO/HIRc, but minimally transcribed in CHO/neo cells. PTP-3 shared 65% of homology with RPTPg, a known mouse protein tyrosine phosphatase, and PTP-3 mRNA levels were 7-fold higher in CHO/HIRc compared to control CHO/neo cells. Northern blot hybridization of poly-A RNA using the 503-bp probe revealed that presence of a homologous 4.4Kb full-length transcript in CHO/HIRc cells. The addition of insulin to serum-free cultured CHO/HIRc further increased PTP-3 mRNA levels in a dose and time dependent manner. Site-directed mutagenesis of the tyrosine kinase domain of the insulin receptor abolished PTP-3 gene expression. Similarly mutation of the juxtamembrane tyr-960 involved in signaling via IRS(s) reduced PTP-3 mRNA levels, when compared to wild type CHO/HIRc. Finally, transfection of CHO cells with other tyrosine kinase-signaling receptors, like IGF-1 or EGF- receptors, was not associated with an increase in PTP-3 levels above control CHO/neo, indicating that the identified sequence was specifically associated with the insulin receptor and not to the presence of other tyrosine kinase receptors. **Conclusions:** PTP-3 is a novel mediator in the insulin signaling cascade that shares a high degree of homology with known tyrosine phosphatases.

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INVOLVEMENT OF $p125^{FAK}$ IN THE INSULIN-MIMETIC SIGNALING OF PHOSPHO-INOSITOL-GLYCAN COMPOUNDS IN ADHERENT/NON-ADHERENT ADIPOCYTES

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Aims: Phosphoinositolglycan compounds (PIG), the polar core glycan head groups of extracellular glycosyl-phosphatidylinositol (GPI)-anchored plasma membrane proteins, have been demonstrated to potently (EC_{50}=0.3-2 μM) stimulate glucose transport (to up to 90±15% of the maximal insulin effect) by induction of IRS-1 tyrosine phosphorylation (up to 7.5±1.3-fold over basal) in the absence of activation of the insulin receptor tyrosine kinase in isolated rat adipocytes and 3T3-L1 adipocytes maintained in suspension. In search for a PIG-dependent candidate tyrosine kinase for IRS-1 we studied the involvement of the $p125^{FAK}$, since this foecal adhesion kinase seems to act as a signaling platform for IRS-1 and non-receptor tyrosine kinases, like $pp60^{src}$. **Results**: Clustering of ß1-integrin by fibronectin plus non-blocking monoclonal anti-ß1 antibodies in isolated rat and non-adherent 3T3-L1 adipocytes inhibited IRS-1 tyrosine phosphorylation and glucose transport activation by 20 μM PIG by about 70±14 and 55±10%, respectively, vs. incubation of the cells with poly-L-lysine plus anti-ß3 antibodies (19±7 and 11±5%). PIG (0.1-20 μM) concentration-dependently induced tyrosine phosphorylation of $p125^{FAK}$ to up to 4.5±0.8-fold and of paxillin to up to 6.8±1.3-fold as well as association of IRS-1 with $p125^{FAK}$ to up to 3.2±0.5-fold over basal in these cells. In adherent 3T3-L1 adipocytes these PIG effects were considerably diminished (by 65-85% vs. non-adherent). Introduction of a peptide corresponding to the FAK major autophosphorylation and Src docking site, Tyr-397, into rat adipocytes by electroporation did not significantly impair PIG-induced $p125^{FAK}$ and paxillin tyrosine phosphorylation as well as association of IRS-1 and $p125^{FAK}$ but reduced PIG-induced IRS-1 tyrosine phosphorylation and glucose transport (to up to 1.9±0.5-fold over basal and 24±8% of the maximal insulin effect). **Conclusions**: Thus, both ß1-integrin clustering and PIG action may induce tyrosine phosphorylation of IRS-1 by causing $p125^{FAK}$-mediated recruitment of IRS-1 and a non-receptor tyrosine kinase. This implicates similar mechanisms of cross talk of extracellular matrix proteins and of extracellular PIG (and potentially GPI) molecules with (metabolic) insulin signaling based on the integrin system and dependent on the cell architecture.

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INSULIN SIGNALLING IN IRS-1-DEFICIENT BROWN ADIPOCYTES: REQUIREMENT OF IRS-1 FOR MITOGENESIS AND LIPID SYNTHESIS.

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Aims: In this study we have investigated the role of IRS-1 in insulin-mediated signalling regarding mitogenesis and lipid metabolism in fetal brown adipocytes. **Materials and Methods:** Immortalized brown adipocyte cell lines generated from fetuses of IRS-1$^{-/-}$, IRS-1$^{+/-}$ and wild type mice were studied. Immunoprecipitations, Western and Northern blot analysis, PI3-kinase activity, insulin receptor autophosphorylation assay, Red Nile fluorescence, ^{3}H-Thymidine incorporation and the cell cycle phases were assayed. **Results:** Under growing conditions IRS-1$^{-/-}$ brown adipocytes maintained the expression of adipogenic (fatty acid synthase) and thermogenic (uncoupling protein-1) markers. The lack of IRS-1 concurred with a 20.5 ± 5.1% increase in IRS-2 protein expression. Insulin-induced tyrosine phosphorylation of IRS-1 was absent in IRS-1$^{-/-}$ brown adipocytes, while IRS-2 tyrosine phosphorylation increased by 3-fold. Consequently, insulin-stimulated IRS-1$^{-/-}$ cells showed increased IRS-2-associated αp85 subunit of PI 3-kinase and IRS-2-associated PI 3-kinase activity. Activated Akt/PKB was decreased by 92 ± 4.7% in insulin-stimulated IRS-1$^{-/-}$ cells, whereas p70s6k remained unchanged. Insulin treatment for 24 h doubled the cytosolic lipid content in IRS-1$^{+/+}$ brown adipocytes, but failed to increase the cytosolic lipid content in IRS-1$^{-/-}$ cells. Regarding mitogenesis, IRS-1$^{-/-}$ cells did not respond to insulin in inducing SHC tyrosine phosphorylation and MAPK activation. Moreover, insulin treatment for 48 h of IRS-1$^{-/-}$ brown adipocytes did not increase ^{3}H-Thymidine incorporation and the entry of cells in the S+G_2+M phases of the cell cycle. **Conclusions:** **1-** IRS-1/PI 3-kinase/Akt activation is an essential requirement for insulin sstimulation of lipid synthesis in brown adipocytes. **2-** IRS-1/MAPK pathway mediates insulin-induced mitogenesis in brown adipocytes.

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SPECIFIC DEGRADATION OF IRS-1 BY HYPERINSULINEMIA: REGULATION BY PHOSPHORYLATION.

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Aim: We have demonstrated that IRS-1 protein expression is markedly reduced in adipocytes from NIDDM subjects in comparison with both healthy and IDDM individuals. In this study 3T3-L1 or healthy human adipocytes were treated for different periods of time with insulin to investigate the possible mechanisms for the downregulation of IRS-1. **Methods:** Adipocytes were incubated in the presence of insulin with or without the addition of different inhibitors. Glucose uptake, western blotting and kinase activities were measured at different times. **Results:** Incubation of the adipocytes with 1uM insulin induced a fast electrophoretic mobility shift of IRS-1 caused by phosphorylation of the protein in serine/threonine sites. After 24 hs of insulin treatment, IRS-1 protein level and the insulin-stimulated glucose transport were reduced. To further investigate the mechanism(s) adipocytes were incubated for 1.5, 5 or 24 hs in the presence of insulin and different protein kinase inhibitors. Wortmannin blocked the mobility shift of IRS-1 suggesting that PI 3-kinase mediates IRS-1 serine phosphorylation, increased the insulin-induced tyrosine phosphorylation of IRS-1 and later blocked its degradation. In contrast, a PKB inhibitor and different PKC inhibitors had no effect. Treatment of the cells with vanadate in the presence of insulin inhibited the tyrosine dephosphorylation of IRS-1 and partially the degradation of the protein. In contrast, incubation with okadaic acid (OA) increased the serine/threonine phosphorylation of IRS-1 and its degradation. Interestingly, the addition of the proteasome inhibitor lactacystin in combination with insulin or OA blocked IRS-1 degradation. **Conclusions**:1)Treatment of adipocytes with insulin "in vitro" mimics the characteristics of adipocytes from NIDDM patients (low IRS-1) 2) Wortmannin but not a PKB or PKC inhibitors blocked insulin-induced degradation of IRS-1. 3) PI3-kinase participates as a negative regulator of IRS-1 by promoting its serine/threonine phosphorylation and inducing its degradation. 4) Regulation of serine/threonine vs tyrosine phosphorylation modulates IRS-1 degradation and insulin resistance in adipocytes.

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INSULIN SIGNALLING ABNORMALITIES IN THE HEART OF INSULIN-DEFICIENT DIABETIC RATS: EFFECTS OF ISLET TRANSPLANTATION.

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Insulin-deficient diabetes is characterised by impaired insulin action in the heart, leading to altered cardiac metabolism and function. **Aims.** To investigate insulin signalling reactions in the myocardium of streptozocin (STZ)-diabetic rats *in vivo* and to assess the effect of diabetes treatment by islet transplantation. **Materials and Methods.** Control (C), untreated STZ-diabetic (D), and STZ-diabetic rats treated by islet transplantation (T) were studied in the basal state or 30 min after insulin injection (20 U/rat, i.p.). Tyrosine phosphoproteins, total amounts of various insulin signalling proteins, and activation of PKB and Erk1/2 were detected by immunoblotting with specific antibodies. **Results.** Insulin-stimulated tyrosine-phosphorylation of the insulin receptor was increased by 45% in D compared to C, with an associated 35% increase in the insulin receptor protein content. Phosphorylation of the IRS proteins after insulin stimulation was similarly augmented by 50% in D, with a more pronounced increase in IRS-2 than IRS-1 phosphorylation. Noticeably, IRS-2 protein content was increased by 64%, whereas IRS-1 content was decreased by 41% in D compared to C. Insulin-stimulated association of p85 with IRS proteins was also increased in diabetes, with a greater extent of p85 association with IRS-2 than IRS-1. Unexpectedly, PKB and p70S6-kinase expression and insulin-induced activation were not increased in diabetic heart. All observed changes in insulin signalling in diabetic rats were corrected by islet transplantation. No differences in Shc and Erk1/2 content or Erk1/2 activation were found in C, D, and T. **Conclusions.** (i.) Specific insulin signalling reactions are differently impaired in the diabetic heart; (ii.) IRS-1 and IRS-2 show a distinct pattern of dysregulation; (iii.) there appears to be a defect in the signal transduction from proximal (IRS and PI 3-kinase) to downstream (PKB, p70S6-kinase) steps; and (iv.) islet transplantation effectively corrects signalling abnormalities potentially responsible for cardiac dysfunction in diabetes.

OP 42
Drugs: Benefits and Side-Effects

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DRUG-INTERACTION BETWEEN ENALAPRIL AND GLIBENCLAMIDE MIGHT LEAD TO HYPOGLYCEMIA

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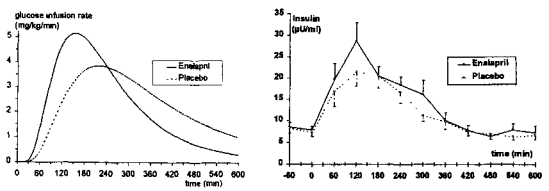
Aims: A higher risk of hypoglycaemic episodes has been reported repeatedly in diabetic patients treated with ACE-inhibitors. We investigated if these findings are at least partly due to a drug interaction between sulfonylureas and ACE-inhibitors. **Materials and Methods:** 9 healthy volunteers (6 male, age 27±3 years (mean±SD), BMI 23±2 kg/m²) received 5 mg Enalapril (ENA) or placebo o.d. over 3 days, resp. in this double-blind cross-over study. At the fourth day of both study periods a euglycemic clamp was established. At each experiment, the volunteers received 3.5 mg Glibenclamide (GLIB) and additionally 10 mg ENA or placebo. Glucose infusion rates necessary to keep blood glucose constant were monitored the subsequent 600 min. **Results:** ENA led to a significant increase of the early metabolic effect of GLIB ($GIR\text{-}AUC_{0\text{-}180}$ 495±228 vs. 361±100 mg/kg/180min, p=0.012); overall metabolic activity was comparable. There was a tendency to a higher and earlier effect with ENA (GIR_{max} 5.2±1.9 vs. 4.1±1.3 mg/kg/min, p=0.095; t_{max} 155±42 vs. 196±48 min, p=0.064; figure). Insulin and C-peptide concentrations were in accordance with these findings (Insulin-$AUC_{0\text{-}180}$ 3759±1518 vs. 3125±1634 µU/ml/180min, C-peptide-$AUC_{0\text{-}180}$ 814±172 vs. 618±677 ng/ml/180min, n.s.). ENA led to a non-significant trend towards a lower rate constant of elimination (0.0064 vs. 0.0072/min, p=0.104) and a higher $t_{50\%}$ (111 vs. 100 min, p=0.114) for GLIB. **Conclusions:** These results suggest that co-administration of ACE-inhibitors and sulfonylureas induces a more pronounced hypoglycaemic effect early after intake. This drug interaction might contribute to the increased risk of hypoglycaemia in diabetic patients treated with ACE-inhibitors.



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ROSIGLITAZONE IMPROVES GLYCEMIC CONTROL WITHOUT ADVERSELY AFFECTING CARDIAC FUNCTION IN TYPE 2 DIABETES

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Aims: RSG is a potent thiazolidinedione that reduces insulin resistance and improves glycemic control in patients with type 2 diabetes mellitus (T2DM). In animal studies, all thiazolidinediones have produced cardiac hypertrophy. Doses of RSG >100 times the human therapeutic dose produced reversible increases in cardiac weight in rats and dogs without significant histopathological effects. This study investigated the effect of long-term treatment with the maximal therapeutic dose of RSG on cardiac structure and function in patients with T2DM and also assessed changes in glycemic control. **Materials and Methods:** Two hundred three patients were randomly assigned to receive glyburide (GLB) (mean dose 10.5mg/d) or RSG (4mg/bd) for 52 weeks. Changes in left ventricular mass index (LVMI), ejection fraction (EF), and left ventricular end diastolic volume (LVEDV) were assessed by M-mode echocardiogram. Glycemic control was assessed by HbA1c and fasting plasma glucose (FPG). **Results:** RSG and GLB had small, clinically insignificant effects on LVMI. No patient shifted from a low or normal LVMI at baseline to a high LVMI on therapy. EF did not change in either treatment group. Both groups had clinically insignificant increases in LVEDV. RSG significantly reduced diastolic blood pressure (2.3 mm Hg; P=0.0016) as assessed by 24-hr ambulatory monitoring. The mean change from baseline in HbA1c was –0.9±1.4 in both the GLB and RSG groups. The change from baseline in FPG at week 52 was –3.11mmol/L in the GLB group compared with –3.61mmol/L in the RSG group. **Conclusions:** Treatment with RSG 4mg/bd for 52 weeks improved glycemic control and did not adversely affect cardiac structure or function in patients with T2DM.

	LVMI (g/m²)		
	Baseline (N)	Week 28 (N)	Week 52 (N)
Glyburide	75±19.0 (97)	76±17.2 (71)	78±16.5 (63)
RSG 4mg/bd	75±20.2 (104)	78±17.9 (72)	79±17.9 (58)
	HbA1c (%)		
Glyburide	9.5±1.59 (99)	8.1±1.46 (77)	8.4±1.46 (68)
RSG 4mg/bd	9.1±1.68 (104)	8.2±1.60 (74)	8.0±1.73 (61)

All values are mean ± SD.

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EFFICACY AND SAFETY OF CERIVASTATIN/BEZAFIBRATE COMBINATION THERAPY FOR DYSLIPIDAEMIA

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Aims: To evaluate the efficacy of cerivastatin (C)/bezafibrate (B) combination therapy on blood lipid levels in non-diabetic patients with dyslipidaemia. The study is relevant to type 2 diabetes as the condition is often associated with a poor triglyceride (TG)/HDL-C profile, inadequately treated with drugs such as HMG-CoA reductase inhibitors. **Materials and Methods:** This was a randomized, multinational, double-blind study. On entry, all patients had (1) an LDL-C level ≥4.12 mmol/l or (2) an LDL-C level ≥3.35 mmol/l and either a history of coronary heart disease or two or more cardiovascular risk factors. In Phase A, all patients initially received placebo for 8 weeks. They then entered Phase B, which was split into two parts: Period 1 and Period 2. In Period 1, patients received C 0.3 mg/day or B 400 mg/day for 8 weeks. This was followed by Period 2, in which patients underwent a second randomization. This determined whether they kept receiving their initially assigned drug for a further 8 weeks or received C/B combination therapy for an additional 8 weeks. **Results:** Percentage changes in efficacy variables from baseline in the ITT population:

	Period 1		Period 2		
LS means (SE)	B (*n* = 125)	C (*n* = 241)	B (*n* = 119)	C (*n* = 116)	Combin. (*n* = 116)
LDL-C*	–19.2 (1.2)	–35.0 (0.9)	–21.1 (1.3)	–34.0 (1.4)	–46.2 (1.3)
Total-C*	–14.4 (0.9)	–24.6 (0.7)	–15.7 (1.0)	–23.5 (1.0)	–29.2 (1.0)
HDL-C*	+22.5 (1.4)	+8.1 (1.0)	+25.1 (1.6)	+11.6 (1.7)	+33.8 (1.6)
TGs*†	–35.8 (2.1)	–13.4 (1.6)	–40.3 (1.8)	–13.5 (1.9)	–44.1 (1.9)

*Pairwise comparison between groups: $P < 0.01$; †except for B vs. combination, which was not significant. Both drugs were well tolerated. Combination therapy did not markedly increase the potential for elevation of hepatic enzymes. **Conclusions:** Combined C/B therapy is more effective than either drug used alone for treating patients with dyslipidaemia. The combination may have utility for decreasing triglyceride levels and increasing HDL-C levels in patients with type 2 diabetes.

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EFFICACY AND SAFETY OF CERIVASTATIN IN THE TREATMENT OF PATIENTS WITH TYPE 2 DIABETES AND HYPERCHOLESTEROLAEMIA

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Aims: To investigate the efficacy and safety of the potent statin, cerivastatin, in the treatment of hypercholesterolaemia (LDL-C >3.35mmol/l and triglycerides [TG] <4.56mmol/l) in patients with type 2 diabetes. **Materials and Methods:** This was a multinational, double-blind, placebo-controlled study. A total of 256 patients were randomized to receive cerivastatin 0.1mg/day, cerivastatin 0.3mg/day or placebo, in a 2:2:1 ratio, for 12 weeks. **Results:** See table below for per-protocol population data: B=baseline, E=endpoint; (LS mean % change)

	Placebo (n=45)		Ceri. 0.1 mg (n=101)		Ceri. 0.3 mg (n=106)	
	B	E	B	E	B	E
Total C[1] (mmol/l)	6.39	6.46 (+1.5%)	6.45	5.55 (–13.7%)	6.34	4.84 (–23.5%)
LDL-C[1] (mmol/l)	4.29	4.29 (+0.6%)	4.34	3.45 (–20.2%)	4.24	2.80 (–33.8%)
HDL-C[2] (mmol/l)	1.14	1.17 (+3.1%)	1.13	1.19 (+5.7%)	1.15	1.21 (+6.2%)
TG[3] (mmol/l)	2.09	2.17 (+4.5%)	2.12	1.97 (–3.9%)	2.07	1.79 (–12.3%)

[1]$P<0.001$ for difference between the 3 groups; $P<0.05$ for 0.3mg vs 0.1mg; $P<0.05$ for 0.1mg vs placebo. [2]No significant difference between the 3 groups. [3]$P=0.0004$ for difference between the 3 groups; $P<0.05$ for 0.3mg vs placebo; no significant difference between 0.1mg and placebo. There were no significant differences between the groups with regard to HbA_{1c}, adverse events or elevation in liver or muscle enzymes. **Conclusions:** Cerivastatin treatment significantly reduces LDL-C and total cholesterol levels in patients with type 2 diabetes and hypercholesterolaemia. There was a dose-ranging effect, with the cerivastatin 0.3mg/day dose proving particularly effective for reducing hypercholesterolaemia and producing a co-reduction in TG levels. The reduction in TG was particularly impressive, as these were almost normal at baseline. These data support the use of cerivastatin as first-line therapy for treating dyslipidaemia in patients with type 2 diabetes, and thus preventing cardiovascular morbidity and mortality.

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NALOXONE AS A POSSIBLE TREATMENT FOR TYPE II DIABETES MELLITUS?

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Glucose stimulation of the pancreatic mouse beta cell line MIN6 upregulates expression of the opioid precursor prodynorphin mRNA as well as increases the content and secretion of dynorphin. Increased secretion of dynorphin was also seen in rat islets. **Aims:** The purpose of the present study was therefore to investigate the role of dynorphin as a possible autocrine factor in the regulation of glucose-induced insulin secretion. **Results:** Neither dynorphin 1-17 nor dynorphin 1-13 influenced insulin secretion from freshly isolated rat islets. These results were independent of glucose concentration (2.8, 6 or 11 mM) and incubation time (1 h, 3 h, 3 days), and were not due to peptide degradation. Similarly, dynorphin at 10^{-9} to 10^{-12} M did not induce changes in cytoplasmic free Ca^{2+}-concentration ($[Ca^{2+}]_i$) in dispersed islets or in FACS-purified beta cells under similar conditions. Identical findings were obtained using the other prodynorphin-derived peptides Met- and Leu-enkephalin. In contrast, naloxone increased glucose-induced insulin release by rat islets in vitro approximately two-fold following stimulation at 4.5 mM (121±4 vs. 238±22 ng/ml, P=0.0004) and 11 mM glucose (1036±61 vs. 2157±868 ng/ml, P<10-5). The effect was seen following exposure to naloxone in the concentration range 10ng/ml-120mg/ml. No significant effect of naloxone alone was seen in unstimulated or fully stimulated cells (2.8 or 20 mM glucose). These results could explain the beneficial effect of naloxone in hyperandrogenic women in which long-term oral naloxone treatment normalises hyperinsulinaemia and in which the effect of naloxone is assumed to be exerted through abolition of an inhibitory tonus by opioid peptides. **Conclusion:** Since we did not see inhibitory effects of any of the opioid peptides used in the present study, it is possible that naloxone stimulates beta cells directly through an hitherto unknown mechanism. Such action could be the mechanism of action in clinical trials which have demonstrated an improvement of glucose tolerance in type II diabetes mellitus patients. Therefore, naloxone analogues might be useful in the development of secretagogues for the treatment of type II diabetes patients.

OP 43
Hypoglycaemia

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SEVERE HYPOGLYCEMIA IN PEDIATRIC PATIENTS: MULTICENTER STUDY INCLUDING 7883 PATIENTS FROM 65 CENTERS.

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Aims: Severe hypoglycemic episodes are a major obstacle towards the achievement of tight metabolic control of diabetes. Few data on the frequency and on contributing factors in children and adolescents are available.
Materials and Methods: The German Working Group on Pediatric Diabetology has established a prospective, longitudinal computer documentation system for inpatient and outpatient care. Both quality indicators and individual patient documentation are anonymized and transmitted for centralized analysis. For 1998, 65 pediatric centers with a total of 7883 patients participated (mean age: 16.3 years, mean duration : 5.9 years).
Results: Defining severe hypoglycemia as events either requiring outside help or characterized by unconsciousness, 21.8 episodes per 100 patient years were documented during 1998. This figure was stable over the last 4 years of the documentation. However, there was a vast difference among the participating centers, with rates of severe hypoglycemia ranging from 2.0 to 56.1 episodes per 100 patient years. At least one severe hypoglycemic episode was documented in 167 patients. Multiple logistic regression analysis revealed that younger age ($p<0.02$), a longer duration of diabetes ($p<0.01$) and lower HbA_{1c} levels ($p<0.02$) were significantly related to severe hypoglycemic episodes. However, the mean HbA_{1c} differed only slightly between patients with severe hypoglycemia (7.37 ± 1.31 %) and patients without (7.74 ± 1.69 %). In contrast, gender, number of daily insulin injections, dose of insulin per kg of body weight and ratio of rapid acting insulin to intermediate/lente insulin did not contribute significantly.
Conclusions: Severe hypoglycemic episodes are frightening for families and patients with type 1 diabetes. According to this structured multicenter documentation, pediatric patients in Germany will experience about one such episode every 5 years. Risk factors are young age, long duration of diabetes, and low HbA_{1c}, however the effect of metabolic control is overall limited.

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BIOLOGICAL AND BEHAVIOURAL DETERMINANTS OF MILD HYPOGLYCAEMIA IN INTENSIVELY TREATED IDDM PATIENTS

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Background: mild hypoglycaemic episodes play an important role in the pathogenesis of impaired hypoglycaemia awareness. **Aims:** to identify determinants of a high mild hypoglycaemia frequency in intensively treated IDDM patients. **Material and methods:** we studied 31 IDDM patients (19 men, 12 women, age 32.0 ± 8.6 yrs., diabetes duration 14.3 ± 8.3 yrs, HbA1c 7.2 ± 0.7 %). Mild hypoglycaemia frequency was defined as all home blood glucose monitoring (HBGM) readings < 3.5 mmol/L recorded in a standardised 6-week HBGM diary. Potential determinants studied were: age, BMI, gender, self-reported hypoglycaemia unawareness, diabetes duration, daily insulin dosage, HbA1c and average and standard deviation (SD) of the 6-week HBGM readings (biological variables), average and SD of alcohol and carbohydrate intake, performance of strenuous physical activity, eating behaviours and psychological distress (behavioural variables). Alcohol and carbohydrate intake were assessed from 3-day food diaries, strenuous physical activity from 7-day physical activity diaries. Psychological distress was measured with the SCL-90 questionnaire and eating behaviour with the Dutch Eating Behaviour Questionnaire (DEBQ), with subscales for restrained, emotional and external eating. **Results:** hypoglycaemia frequency ranged from 0 to 41 episodes (18.7 ± 11.0). In univariate regression analyses the following determinants were identified: HBGM SD (β 0.6, p=0.001), self-reported hypoglycaemia unawareness vs awareness (0 vs 1) (β -0.5, p=0.003) and diabetes duration (β 0.5, p=0.008). A trend was found for BMI (β -0.3, p=0.1), performance vs no performance of strenuous physical activity (1 vs 0) (β 0.3, p=0.06) and external eating behaviour (β -0.3, p=0.1). Average HBGM and hypoglycaemia frequency were related only after adjustment for HBGM SD (β -0.6, p=0.001). Hypoglycaemia frequency was not related to HbA1c. **Conclusion:** blood glucose variability rather than glycaemic control per se is an important determinant of a high mild hypoglycaemia frequency in intensively treated IDDM patients. Additional determinants are hypoglycaemia unawareness, diabetes duration, external eating behaviour, BMI and performance of strenuous physical activity. The behavioural factors are of interest as they may be open to intervention.

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ACUTE CONTRIBUTION OF INCREASE IN PLASMA CORTISOL TO RESPONSES TO HYPOGLYCEMIA IN HUMANS.

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To establish the acute, specific contribution of plasma cortisol (CORT) responses to hypoglycemia (H), 8 normal subjects (4M, 4F, age 26±1 yrs, BMI 23±0.2 kg/h^2) (mean±SEM) were studied during stepped H (plateau plasma glucose, PG, of 90, 78, 66, 54, 42 mg/dl), induced by hyperinsulinemic-hypoglycemic glucose clamp, on 3 occasions: placebo (Study I, SI), suppression of CORT release by oral metyrapone (Study II, SII), or suppression of CORT as in S2 + replacement of i.v. CORT to reproduce the responses of SI (Study III, SIII). In SII, lack of increase in CORT (9.6±0.5 vs 25±2 of SI, µg/dl, p<0.05) resulted in greater increase in early phase in adrenaline (2.62±0.3 vs 1.91±0.2 nmol/l) and noradrenaline (2.25±0.3 vs 1.21±0.15 nmol/l) (p<0.05), and glucagon to H (123±19 vs 107±13 pg/ml, p<0.05), but maximal responses were superimposable (p=NS). Maximal responses of growth hormone were greater in SII vs SI (91±17 vs 64±15 ng/ml, p<0.05). Glucose counterregulation (CR) was not impaired in SII vs SI as indicated by similar needs for exogenous glucose during the clamp (p=NS). Autonomic symptoms were greater in SII vs SI (score 11.4±1 vs 9.8±0.8), as well as neuroglycopenic symptoms (6.9±0.5 vs 4.6±0.4) (p<0.05). Cognitive function (sum of z scores of 12 psichometric tests) deteriorated more in SII than in SI (-42±7 vs 29±4, p<0.05). Replacement of CORT increase during H in SIII (26±3 µg/dl, p=NS vs SI) normalized the early responses of glucagon, adrenaline/noradrenaline, growth hormone, symptoms, and greater impairment in cognitive function to H of SII (p=NS vs SI). Conclusions: increase in plasma CORT during short-term H, 1)has no acute CR effects, but 2)modulates the responses of CR hormones and autonomic symptoms, and, 3)protects brain function during H.

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EFFECT OF CORTISOL REPLACEMENT ON RESPONSES TO HYPOGLYCEMIA IN ADDISON S DISEASE.

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To establish the effect of cortisol (CORT) replacement on responses to hypoglycemia (H) in autoimmune Addison s disease, 6 Addison patients (Add-pts) were studied on 2 random occasions, 1 month apart, during stepped H (plasma glucose, PG, 90, 78, 66, 54, 42 mg/dl, hyperinsulinemic-hypoglycemic clamp technique) to establish responses of counterregulatory hormones, symptoms (S) and cognitive function (CF, 12 psichometric tests). On one occasion (#1), Add-pts were studied after 16 hour withdrawal of oral cortisone (CORT deficiency during H), on the other (#2) after i.v. replacement of CORT to match plasma CORT levels before/during H, of 10 normal subjects (N) studied as controls. In #1, responses of adrenaline (Adr, 2.21±0.6 vs 6.7±1.6 nmol/l) (mean ± SEM) and glucagon (IRG, 7±2 vs 55±8 pg/ml) were blunted (p<0.05), but noradrenaline (Noradr) greater (3.5±0.7 vs 2.9±0.3 pg/ml); total autonomic S were similar to N (score 12.4±2.8 vs 13.7±2.3, p=NS), as result of lower autonomic adrenergic S (heart pounding, tremor, anxiety, 5.2±1.5 vs 7.7±1.4) but preserved autonomic cholinergic S (sweating, hunger, paresthesias, 5.5±1.4 vs 7.4±0.7); neuroglycopenic S were greater than N (12±1.5 vs 8±1.8 pg/ml); CF was severily impaired (sum of z scores, -53.7±8 vs 28.7±6, p<0.05). In #2, normalization of CORT did not improve Adr, neither reduced Noradr responses, neuroglycopenic or autonomic S (p=NS vs #1), but normalized IRG responses (63±6 pg/ml). However, CF deteriorated less vs #1 (-32±7), and did no longer differ from N (p=NS). Conclusions: Add-pts exibit reduced Adr responses to H that are not corrected by normalization of CORT in peripheral plasma, but are H aware, due to autonomic cholinergic S. Deficient Adr is likely responsible for blunted IRG responses. CORT is critical to protect brain from deteriorated CF during H.

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IMPROVEMENT IN COGNITIVE DYSFUNCTION BY HIGH BLOOD LACTATE LEVELS DURING HYPOGLYCAEMIA IN TYPE 1 DIABETIC PATIENTS.

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Aims: In normal subjects, an acute elevation in plasma lactate levels after symptomatic responses to hypoglycaemia have developed prevent further deterioration in cognitive function. **Methods:** To determine whether the same occurs in diabetic patients we have studied 7 male type 1 diabetic individuals (age 32±2 yrs, disease duration 18±2 yrs, HbA1c 8.7±0.2 %) on two different occasions during a hypoglycaemic-hyperinsulinemic clamp (insulin infusion 1.5 mU/kg/min, plasma glucose reduced stepwise from 95 to 70, 60 and 50 mg/dl every 40 minutes). Lactate (30 µmol/kg/min) or saline were infused in random order immediately after the onset of symptomatic responses to hypoglycaemia (sweating, tremor, anxiety). **Results:** In both studies adrenaline (3.4±0.1 vs. 3.5±0.1), noradrenaline (3.2±0.1 vs. 3.2±0.1) cortisol (3.2±0.1 vs. 3.2±0.1) and growth hormone responses (3.6±0.1 vs. 3.6±0.1 mmol/l) started at similar plasma glucose levels. Similarly, autonomic and neuroglycopenic symptoms were experienced in both studies at similar glucose thresholds (3.2±0.1 vs. 3.2±0.1, and 3.1±0.1 vs. 3.2±0.1 mmol/l, respectively). Lactate infusion significantly reduced counterregulatory (adrenaline peak 3.2±0.3 vs. 1.4±0.4 nmol/l, p=0.001, AUC 1.21 ± 0.2 vs. 0.58 ± 0.1 nmol/l/min, p=0.03; cortisol AUC= 375±44 vs. 248±28 nmol/l/min, p=0.04) and symptomatic (autonomic score = 6.3±1.6 vs. 1.3±0.05; p=0.02; neuroglycopenic score = 5.7±1.1 vs. 2.3±0.5; p=0.04) responses to hypoglycaemia. Cognitive function (4-choice reaction time) deteriorated at similar glucose thresholds (3.2±0.2 vs. 3.4±0.2 mmol/l, p=0.23). However, at glucose nadir (2.8 mmol/l) no further deterioration was observed during saline infusion while cognitive function significantly improved with lactate infusion (Δmsec= 0.2±0.4 vs -38±0.2, p=0.0001). **Conclusion:** in type 1 diabetic patients acute elevation in plasma lactate concentration prevents counterregulatory and symptomatic responses to progressive hypoglycaemia as already observed in normal subjects. However, a better recovery of cognitive function seems to occur in diabetic patients.

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A ROLE FOR PARAVENTRICULAR HYPOTHALAMIC α-ADRENOCEPTORS IN THE COUNTERREGULATION TO HYPOGLYCEMIA

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Hypoglycemia-Associated Autonomic Failure (HAAF), an experimental model for *hypoglycemia unawareness*, is characterized by attenuation of the counter-regulatory autonomic and hormonal responses to hypoglycemia. It is generally induced by multiple hypoglycemic episodes caused by consecutive infusions of insulin. **Aims:** In relation to the known involvement of the paraventricular nucleus of the hypothalamus (PVN) in energy homeostasis, the aim of the present study was to investigate the role of the noradrenergic neurotransmission in the PVN in the counterregulatory responses to hypoglycemia. **Materials and methods:** All tests were performed in freely-moving rats, cannulated for stress-free blood sampling and PVN infusions. Selective α_1- or α_2-adrenoceptor antagonists (prazosin and yohimbine) or vehicle were administered into the PVN of rats which were subsequently subjected to insulin-induced hypoglycemia (125 mU·min^{-1}·kg^{-1} insulin for 90 minutes). Blood samples were frequently collected to measure changes in counterregulatory responses. **Results:** Insulin-induced hypoglycemia (controls) significantly increased plasma glucagon, adrenaline and noradrenaline levels (peak values 160 ± 14, 1598 ± 611 and 350 ± 27 pg/ml (average ± SEM); $p < 0.05$). Prazosin (α_1) significantly decreased the adrenaline (785 ± 223 *vs* 1598 ± 611 pg/ml) and noradrenaline responses (270 ± 17 *vs* 350 ± 27 pg/ml). Yohimbine (α_2) decreased the adrenaline response (860 ± 287 *vs* 1598 ± 611 pg/ml). **Conclusions:** The data reveal that noradrenergic transmission in the PVN may play a role in the counterregulation to hypoglycemia. Since the observed decreases are remarkably similar to those found in HAAF, it is suggested that the development of HAAF may be a consequence of a reduction of α-adrenergic neurotransmission in the PVN.

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SEVERE HYPOGLYCAEMIA, COGNITION AND MRI STRUCTURAL ABNORMALITIES YOUNG PATIENTS WITH TYPE 1 DIABETES.

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Protracted severe hypoglycaemia can cause structural and cognitive brain deficits. Severe hypoglycaemia may have a cumulative detrimental effect on cognitive function in people with Type 1 diabetes (IDDM) and strict glycaemic control increases the risk of exposure to severe hypoglycaemia. **Methods:** Seventy-four young normotensive subjects[(mean ± SD) age 29±6 yrs, age at diagnosis 10 ± 4 yrs, duration 19 ± 6 yrs, BP 119/73 ± 12/8 mmHg, HbA1c 8.1 ± 1.0%] without microvascular disease, or minimal BDR only, participated in a cross-sectional study exploring the relationship between frequency of severe hypoglycaemia, cognitive function [NART, WAIS-R sub-tests, Inspection Time, Choice Reaction Time, Borokowski Word Fluency, PASAT] and brain structure through detailed magnetic resonance imaging (MRI). Participants developed Type 1 diabetes before attainment of full intellectual maturity, had no previous CNS pathology and variable exposure to severe hypoglycaemia[(mean ± SD) 17 ± 35 episodes, 26% no episodes, 27% 1-4 episodes, 17% 5-9 episodes, 30% > 10 episodes] as assessed by retrospective questionnaire. **Results:** Neuropsychological test scores, including comparison of NART and current performance IQ measures, did not correlate to severe hypoglycaemia exposure nor did MRI brain volumes and estimates of brain atrophy. An early onset age of Type 1 diabetes was associated with a lower hippocampal-amygdala volume/whole brain volume ratio, implying relative atrophy of medial temporal lobe structures (r=0.359, p=0.007, Pearson's). **Conclusions:** No significant cognitive or brain structural abnormalities were identified in association with severe hypoglycaemia in patients whose diabetes was diagnosed during childhood and was longstanding. These observations are reassuring both to patients and clinicians and imply that recurrent severe hypoglycaemia, unless particularly protracted, does not have a significant impact on central nervous system function over the timescale of diabetes examined by the study.

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A COMPARISON OF THE METABOLIC AND CARDIAC ELECTROPHYSIOPHYSIOLOGICAL CHANGES DURING EXPERIMENTAL AND CLINICAL HYPOGLYCAEMIA.

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Aims Abnormal ventricular repolarization develops during insulin induced hypoglycaemia, which in other conditions can initiate cardiac dysrhythmias. Whethe similar changes occur in patients during clinical episodes is not clear. We have therefore examined the effect of spontaneous nocturnal hypoglycaemia upon the QTc interval, a marker of ventricular repolarization, in a group of tightly controlled patients, and compared these data with changes in ventricular repolarization during experimental hypoglycaemia in normals.

Methods and Results 17 healthy males (mean+/-SEM age 27.7+/-1.6yr, BMI 23.5+/-kg/m2) underwent identical hyperinsulinaemic hypoglycaemic clamps with blood glucose maintained at 5mM for 30min followed by 30 min at 2.5mM. We made regular measurements of plasma potassium, counterregulatory hormones and QTc interval. During each study potassium fell significantly (Initial and final level, 4.2±0.3 to 3.4±0.2mM, P<0.001), adrenaline increased (Initial and final level 0.19±0.01, to 4.87±0.48nM,P<0.001), and QTc increased from baseline (by 79±6ms, P<0.001). Of 22 Type I diabetic patients monitored overnight, 7 (mean age 40.8±5.1 y, HbA_{1c} 7.2±0.55%) became spontaneously hypoglycaemic (blood glucose < 2.5mM). During these episodes, glucose reached 2.2±0.08mM, potassium 3.61±0.1mM, p=ns, (mean overnight value 3.68±0.04mM) and serum adrenaline reached , 0.34±0.14nM, p=ns (mean overnight adrenaline, 0.19±0.03nM). Mean QTc was unchanged (delta +4±4ms although one patient had an increase of 20ms).

Conclusions Our data show that patients with tight control exhibit neither increases in adrenaline or increases in QTc during spontaneous nocturnal hypoglycaemia. They support the hypothesis that abnormal cardiac repolarization during experimental hypoglycaemia is caused by sympathoadrenal activation. They also suggest those at greatest risk may be patients with relatively short duration of diabetes and poor glycaemic control, who mount brisk sympathoadrenal responses.

OP 44
Nephropathy II

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RISK FACTORS FOR PROGRESSION OF NEPHROPATHY IN TYPE 1 DIABETIC SUBJECTS IN EUROPE.

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Aims: To assess the risk factors for the early and late progression of diabetic nephropathy (DN) in 1919 type 1 subjects from 27 centres participating in the EURODIAB Prospective Complications Study. **Materials and Methods**: Albumin excretion rate (AER) was calculated from a timed 24-hour urine collection at base-line and after an average of 7.4 years follow-up using an immunoturbidimetric method for albumin measured in the same central laboratory. Baseline serum lipids, lipoproteins and HbA_{1c} were also centrally measured. **Results:** Of the 1,135 subjects normo-albuminuric (AER <20µg/min) at baseline, 14.7% progressed to microalbuminuria (AER 20-200µg/min). After adjustment for baseline AER, factors significantly associated with early progression were HbA_{1c} (p<0.0001), triglyceride (p<0.0001), LDL-cholesterol (p<0.0007), HDL-cholesterol (-ve) (p<0.002), and waist/hip ratio (WHR) (p<0.01). There was no evidence for a threshold in the relationship between HbA_{1c} and early progression. Progressors had a higher frequency of retinopathy at baseline (51%) than non-progressors (36%) (p<0.001). Baseline BP did not predict progression. Of the 359 subjects with microalbuminuria at baseline, 14.2% progressed to macroalbuminuria (AER>200µg/min) and 35.4% regressed to normoalbuminuria. The baseline mean AER in the progressors, non-progressors and regressors was 65, 45, 38µg/min respectively (p<0.0001). The prevalence of retinopathy was significantly lower in the regressors (47%) compared with non-progressors (65%). After adjustment for baseline AER, only HbA1c significantly predicted late progression (p<0.001). **Conclusions:** Besides poor glycaemic control adverse lipid patterns and increased WHR, which are features of insulin resistance, are major risk factors for the early stages of DN in type 1 subjects. These findings suggest a possible role for insulin resistance in the pathogenesis of DN.

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RISK FACTORS FOR DEVELOPMENT OF MICROALBUMINURIA IN TYPE 1 DIABETIC PATIENTS: 10 YEARS OBSERVATION AND FOLLOW-UP

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Aims: To evaluate prospectively putative risk factors for development of incipient and overt diabetic nephropathy in Type 1 diabetes. **Material and Methods:** A cohort of all Type 1 diabetic patients with normoalbuminuria (urinary albumin excretion rate (UAER) <30 mg/24 h), age >18 years, duration of diabetes >5 years attending our clinic were identified (n=593). At baseline 44 were excluded because of antihypertensive treatment and 12 were lost to follow up. The remaining 537 patients (mean (SD) age 39 (13) , duration of diabetes 19 (11) years, 279 males), were investigated yearly for the following 10 years. **Results:** The progression of UAER was 7.6 (0.8) % per year (mean(SE)). During follow up 134 patients (25%) progressed to micro- or macroalbuminuria (>30 mg/24h in 2 out of 3 consecutive samples). Cox multiple regression analysis using baseline values of putative predictors of progression, revealed the following significant predictors of progression from normo- to micro-macroalbuminuria: Log_{10} UAER 2.63 (1.65 to 4.19) (relative risk (95% confidence interval)), haemoglobin A_{1c} (%) 1.13 (1.04 to 1.23), retinopathy (any) 1.90 (1.26 to 2.88) and smoking 1.61 (1.11 to 2.33). whereas sex, duration of diabetes, arterial blood pressure, s-creatinine, height and social class were not included in the final model. If follow up was censored at the onset of any antihypertensive agent, the analysis revealed the same predictors. **Conclusion:** Our study suggests that several potentially modifiable risk factors such as minimal elevation of UAER, poor metabolic control, retinopathy and smoking predicts the development of microalbuminuria in Type 1 diabetic patients.

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MORTALITY AND CAUSES OF DEATH IN PEOPLE WITH TYPE 1 DIABETES ON INTENSIFIED INSULIN THERAPY IN RELATION TO BASELINE NEPHROPATHY

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Aims: To assess standardized mortality ratios (SMR) and causes of death in type 1 diabetic patients on intensified insulin therapy in relation to the degree of nephropathy. Methods: All 3674 patients (insulin treatment before age 31) who had participated in a 5-day inpatient treatment and teaching programme for intensification of insulin therapy between 9/1978 and 12/1994 were followed for 10 ± 3 (mean ± SD) years; 50% were women, age at baseline 27 ± 11 yrs, diabetes duration 11 ± 9 yrs. Patients were divided into 3 groups according to the level of baseline nephropathy (group I, normoproteinuria, n = 1829; group II, microproteinuria, n = 1257; group III, at least macroproteinuria, n = 367, missings n = 117). Results: Vital status and causes of death were available for 3570 (97%) patients; 251 (6,8%) patients had died. The following SMR were calculated by using the respective geographic area (North Rhine Westphalia) as reference population: Men: Nephropathy group I, 2.19 (95% CI 1.54-3.02); group II, 3.18 (2.29-4.3); group III, 11.45 (8.78-14.68); Women: group I, 2.48 (1.54-3.79); group II, 3.51 (2.23-5.27), group III, 26.99 (19.83-35.9). Causes of death for men and women combined: group I (total 58 deaths), cardiovascular 21 (36%), hypoglycaemia 1, ketoacidosis 3, violent deaths 17 (29%), others 16; group II (66 deaths): cardiovascular 25 (38%), hypoglycaemia 2, ketoacidosis 2, violent deaths 14 (21%), others 23; group III (114 deaths), cardiovascular 68 (60%), hypoglycaemia 2, ketoacidosis 5, infections 15 (13%), violent deaths 5 (4%), others 19. Conclusions: The present study reports for the first time standardized mortality ratios and causes of death in relation to nephropathy for persons with type 1 diabetes on intensified insulin therapy.

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Heterogeneous Patterns of the Course of Renal Function in Type 2 Diabetic Patients with Proteinuria

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Aims: to study the course of renal function in Type 2 diabetic patients with proteinuria (D) in relationship with the patterns of renal lesions, glycemic control and blood pressure levels. Methods: to study in 34 proteinuric, hypertensive D: 1) base line glomerular basement membrane (GBM) width and mesangial fractional volume [Vv(mes/glom)] using electron microscopy in renal biopsy from D and 27 non diabetic kidney donors; 2) GFR at base line and every 6 months only in D during 3.5 year period of intensified antihypertensive therapy using Ace inhibitors associated with other compounds. Results: Mean GBM width and [Vv(mes/glom)] values were greater in D than controls, (GBM width, [Vv(mes/glom)]: D vs Controls: 512±86, .30±.08 vs 310±38 .19±.03, $p<0.05$ for both, ANOVA and Bonferroni test). Mean (±S.D.) GFR decreased in D (-3.0±13.0 ml/min/1.73m^2 per year (95%C.I.:-7.71+1.61, $p<0.01$). However the values of change were broadly dispersed in a wide range. Thus %GFR change per year, from base line, (Delta%GFR) was calculated. D who had values below and above the median, were defined Progressors and Nonprogressors, respectively. Number of Progressors did not increase across quartiles of base line AER. Number of Progressors did not increase across quartiles of mean blood pressure (MBP). Odds ratio for becoming Progressor increased across quartiles of HbA1c, particularly as for the highest quartile (HbA1c above 8%). Number of Progressors increased across quartiles of GBM width and [Vv(mes/glom)] values, being 3.56 and 4.79 respectively higher in the fourth quartile (greatest GBM width and [Vv(mes/glom)]) than in the first quartile (lowest values). Conversely Nonprogressors outnumber Progressors in the first quartile of GBM width (Odds ratio: 3.34) and in first quartile of [Vv(mes/glom)] (Odds ratio: 4.28). 6 of 17 Progressors developed end stage renal disease. The median AER significantly increased from 505 to 1876 µg/min ($p<0.05$) in Progressors but not in Nonprogressors (from 387 to 423, n.s.). Conclusions: the hallmark of D with proteinuria, in whom GFR rapidly declines, is given by severe diabetic lesions of glomerular structure. Kidney biopsy identifies proteinuric D, otherwise homogeneous as for altered base line AER with poor clinical prognosis, despite tight blood pressure control. Intensified glycemic control using insulin therapy, beside antihypertensive therapy, may be mandatorely needed to delay GFR decline in this cohort of D.

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PROGNOSTIC IMPLICATIONS OF RETINOPATHY AND A HIGH PLASMA VON WILLEBRAND FACTOR LEVEL IN NIDDM SUBJECTS WITH MICROALBUMINURIA

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Aims: It has been suggested that microalbuminuria in subjects with non-insulin-dependent diabetes mellitus (NIDDM) is heterogeneous with respect to clinical features, renal histology and prognosis. We investigated the prognostic implications of the presence of generalized endothelial dysfunction, as indicated by the presence of retinopathy or high plasma von Willebrand factor (vWf) level, among microalbuminuric NIDDM subjects.

Material and methods: In 173 NIDDM subjects of a population-based cohort, we assessed the urinary albumin-to-creatinine ratio, the plasma vWf level, and the presence of retinopathy. The main outcome was cardiovascular mortality with a mean duration of follow-up of 7.0 (2.1) years.

Results: The age- and sex-adjusted relative risk (95% confidence interval) of cardiovascular mortality, as compared to normoalbuminuric subjects without retinopathy, was 1.0(0.1-7.9) for normoalbuminuric subjects with retinopathy; 1.6(0.4-6.0) for microalbuminuric subjects (albumin-to-creatinine ratio 2.0-30.0 mg/mmol) without retinopathy; and 8.9(2.9-27.5) for microalbuminuric subjects with retinopathy. The age- and sex-adjusted relative risk of cardiovascular mortality, as compared to normoalbuminuric subjects without a high vWf level (>183%), was 1.2(0.3-4.3) for normoalbuminuric subjects with a high vWf level; 2.2(0.6-7.8) for microalbuminuric subjects without a high vWf level; and 10.9(2.8-43.3) for microalbuminuric subjects with a high vWf level. These differences in risk of cardiovascular mortality did not materially change after further adjustment for known duration of diabetes, hypertension, and creatinine clearance, level of glycated hemoglobin and HDL-cholesterol, and presence of ischemic heart disease. Consideration of all-cause instead of cardiovascular mortality somewhat decreased the relative risks associated with the presence of microalbuminuria, but did not diminish the difference in risk of mortality between microalbuminuric subjects with or without retinopathy or a high vWf level.

Conclusions: Among NIDDM subjects with microalbuminuria, the presence of retinopathy or a high plasma vWf level strongly affects the risk of cardiovascular death. This supports the concept that microalbuminuria in NIDDM can occur in the absence or the presence of generalized endothelial dysfunction, and that the latter is a much more 'malignant' condition than the former.

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HIGH LEVELS OF PLASMA VON WILLEBRAND FACTOR ARE NOT ASSOCIATED WITH INCREASED RISK OF CARDIOVASCULAR DISEASE IN TYPE 2 DIABETIC PATIENTS WITH MICROALBUMINURIA

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Aim: To examine whether, as it has been suggested, microalbuminuria in combination with high levels of plasma von Willebrand factor (malignant microalbuminuria) is a stronger predictor for cardiovascular disease than microalbuminuria alone (benign microalbuminuria) in type 2 diabetic patients. **Materials and Methods**: 160 patients with type 2 diabetes mellitus and microalbuminuria were followed for an average of 3,8 (SD 0,3) years. Patients in this subanalysis were divided into two groups according to baseline plasma von Willebrand factor levels below (benign) or above (malignant) the median. Main outcome was all-cause mortality, non-fatal stroke, non-fatal myocardial infarction, coronary artery bypass graft and revascularisation or amputation to legs. **Results**: The two groups were at baseline comparable for HbA_{1c}, fasting levels of s-total-cholesterol, s-HDL-cholesterol and s-triglycerides, gender, known diabetes duration, smoking habits, previous cardiovascular disease and antihypertensive therapy. Systolic blood pressure was higher in the malignant group (mean (SD) 150 (18) versus 145 (19), p=0,04 (Mann-Whitney test)) while diastolic blood pressure was similar (87 (9) versus 84 (11), p=0,10). 15 patients in each group experienced a cardiovascular event during follow-up giving an odds ratio of 0,98 (95% confidence interval 0,41 to 2,31) with benign microalbuminuria, p=0,97) using logistic regression with both systolic and diastolic blood pressure and antihypertensive therapy as covariates. **Conclusions**: Our results do not support the suggestion that the combination of high plasma levels of von Willebrand factor and microalbuminuria is a stronger predictor of cardiovascular outcome than microalbuminuria alone in patients with type 2 diabetes and microalbuminuria.

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DIABETIC NEPHROPATHY IN PATIENTS WITH TYPE 1 DIABETES AND EITHER REGULAR OR IMPAIRED NIGHTLY RHYTM OF BLOOD PRESSURE.

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In patients with type 1 diabetes diurnal rhytm of blood pressure (BP) is often disturbed. It seems likely that the lack of the drop of BP in the night may influence the course of diabetic nephropathy.

The aim of this study was the evaluationb of the course of diabetic nephropathy in patients with stage of microalbuminuria presenting either normal (dippers) or impaired (non-dippers) nighty rhytm of BP.

Materials and Methods: Albumin excretion in the urine (UAER) and glomerular filtration rate (GFR) were measured in 36 patients divided into 2 groups: 1.dippers (the drop of BP in the night above 10% of values in the day), n=23, mean age 41,8 years, mean duration of the diabetes 16,7 years, mean UAER at the onset of the study 71,8 μg/min (22,8-168,4), 2. non-dippers {the drop of BP in the night below 10% values in the day , n=13, mean age 41,8 years, mean duration of the diabetes 17,8 years, mean UAER at the onset of the study 68,4 μg/min (24,7-171,2)}.
During the follow up lasting 7 years UAER was measured every 6 months by the use of RIA method and GFR every year by the use of isotopic method. The metabolic control of diabetes was at least satisfactory in all patients. All patients were treated with ACE inhibitors (enalapril or perindopril) at adequate doses.

Results: Significant decrease of UAER was observed in both groups of patients However, it was more pronounced in dippers group (ΔUAER 30,2 vs 21,5 μg/min, p<0,05). During the follow-up UAER normalized below 20 μg/min in 9 dippers patients and in none from non-dippers group. Moreover, despite normal values of GFR at the end of observationin both groups of patients, in the second one (non-dippers). GFR decreased significantly greater (ΔGFR 0,65 vs 0,38 ml/1,73m2/s.a./month).

Conclusions: It seems likely that besides genetic predispositions, metabolic state of the disease and hypertension, impaired circadian rhytm of BP may influence the progression of diabetic nephropathy.

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PROGRESSION OF RENAL DISEASE IN MACROALBUMINURIC TYPE II DIABETIC PATIENTS WITH AND WITHOUT RETINOPATHY.

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Aims: Since it has been suggested that lack of retinopathy is a poor predictor of diabetic renal disease, the aim of our study was to evaluate the rate of progression of renal damage in macroalbuminuric (albumin excretion rate [AER] > 300 mg/24hr) type II diabetic patients with (D+) and without retinopathy (D-).

Materials and Methods: Twenty-five (22 M/3 F) D+ (mean ±SD: age 57±9 yrs, diabetes duration 17±8 yrs) and 22 (19M/3F) D- (age 54±8 yrs, diabetes duration of 15±8 yrs) were followed-up prospectively for a mean period of 4.1 yrs (range 1-10). GFR (^{51}Cr-EDTA bolus injection), blood pressure (BP) and HbA_{1c} were measured every 6-8 months. **Results :** At baseline HbA_{1c} was similar in the two groups (8.6±2.1% in D+ vs 8.3±2.1% in D-) and remained stable during the follow-up period. GFR at entry was similar in the two groups (79.6 [range 22-132] ml/min x 1.73m^2 in D+ vs 82 [range 16-139] in D-). Rate of decline of GFR (median ±95%C.I) was significantly higher in D+ (-5.87±0.07 ml/min x year ; p < 0.01) than in D- (-1.6±0.09). During the study period, mean BP was similar and did not change in both groups (from 112±8 mmHg to 110±8 in D+ vs from 109±10mmHg to 110±12 in D-). All patients were receiving antihypertensive treatment. AER remained stable during the observation period in both groups (from 1.5 [0.5-5.6] g/24hr to 2 [0.6-6.5] in D+ and from 1.9[0.5-4.6] g/24hr to 2 [0.6-5.5] in D-). In a stepwise multiple regression analysis, the rate of decline of GFR in D+ was significantly associated with mean BP (p<0.001) and with albuminuria (p<0.05) during the follow-up period. HbA_{1c} was not associated with the rate of progression of renal damage in D+. **Conclusions :** The rate of progression of renal disease in macroalbuminuric type II with retinopathy is much faster than that observed in those without retinopathy. Lack of retinopathy is associated with a better renal prognosis, independently of blood pressure levels and metabolic control.

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Genetics and Epidemiology of Type 1 Diabetes

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SIGNIFICANT ASSOCIATION BETWEEN INTEFERON REGULATORY FACTOR 2 (IRF2) GENE AND TYPE 1 DIABETES.

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We have previously shown that activity of the interferon-α (IFNA) - inducible enzyme 2'-5'-oligoadenylate synthetase (2'-5'AS) is significantly elevated in both recent-onset and long-term Type 1 diabetics. This enzyme functions in the innate antiviral defense system to clear the host of infecting viruses. We postulated that variation at genes controlling the antiviral defense system may be involved in predisposition to Type 1 diabetes – for example, by determining a less efficient or aberrant response to endogenous or exogenous viruses. Interferon regulatory factors (IRF) 1 and 2 are transcriptional factors that activate and repress (respectively) IFNA transcription, and therefore may affect 2'-5'AS levels. In previous studies, we have shown that genetic variation at the IRF1 locus is not associated with susceptibility to Type 1 diabetes. **Aim:** The aim of the present study was to determine whether variation at the IRF2 locus influences susceptibility to diabetes. **Methods:** Families with 2 or more Type 1 diabetic children were typed for a microsatellite marker at IRF2 on chromosome 4q35.1, including 256 British, American, and Canadian families from previous linkage studies, and 80 Danish families. **Results:** The 256 families showed no evidence for linkage between IRF2 and diabetes (average gene sharing between 309 pairs of affected siblings = 0.499, compared with expected sharing of 0.50). However, using a family-based association test (AFBAC), the total dataset showed significant differences (p = 0.0082) in IRF2 alleles ***transmitted*** versus ***not transmitted*** from parents to diabetic children. The most common allele showed a higher frequency of transmission to diabetics in the overall dataset, and in each of the 4 separate populations. The largest differences were in the North American heterogeneous populations. **Conclusion:** The presence of genetic association without linkage is considered to be indicative of a common genetic factor that influences risk of disease. We conclude that common genetic variation at IRF2 predisposes to Type 1 diabetes.

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ADDITIONAL HLA GENE ASSOCIATED WITH TYPE 1 DIABETES MAPS TO THE 240 KB REGION NEAR HLA-B.

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Aims: Several studies provided evidence that besides the DQ - DR genes, HLA contains an additional uncharacterised gene associated with type 1 diabetes. Our aim was to investigate its effect independently of the DQ-DR genes and to localise this new gene. **Materials and Methods**: More than 1400 Finnish type 1 diabetes patients and 30,000 control individuals representing Finnish population were studied. They were genotyped for the alleles of HLA-DQB1, -DQA1 and -DRB1 genes. 74 patients and 189 controls, stratified for the DQA1*05 - DQB1*02 / DQB1*0302 - DRB1*0404 genotype were selected. Ten microsatellite markers in the HLA class III and I regions (D6S273, TNFa, C12A, STR MICA, MIB, C125, C143, C245, C3211 and MOGc) as well as alleles of HLA-A and -B genes were studied. **Results**: The strongest diabetes association was observed for the markers in the 240 kb region around HLA-B gene, between markers C12A (OR=3.9) and C143 (OR=3.6). The peak association was found for B39 (OR=4.3, p<0.000004). **Conclusions**: Our data indicate that an additional gene associated with type 1 diabetes is located in the 240 kb region near HLA-B close to the centromeric end of HLA class I.

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ASSOCIATION OF HLA-A GENE WITH RESIDUAL INSULIN SECRETION AT THE ONSET OF TYPE 1 DIABETES MELLITUS

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In most cases type 1 diabetes mellitus concern people with genetic predisposition and the inheritance has multigenes character. **Aims:** Verification of the hypothesis about reciprocal connection of HLA-A alleles and residual insulin secretion in diabetics at the onset. **Materials and Methods:** HLA-A alleles genotyping was performed in 112 children with type 1 diabetes mellitus and in 92 healthy blood donors using sequence specific oligonucleotide hybridisation after PCR amplification (PCR-SSO). Moreover, the level of C-peptide in diabetic children at the onset was estimated by radioimmunoassay. **Results:** The frequency of HLA-A*25 allel was higher in diabetics than in control group. We found this allel in 14% (11/77) diabetics and in 1% (1/92) healthy donors (OR=14, p<0,002). Allel HLA-A*0101 was observed more frequently in control group than in patients with type 1 diabetes mellitus, respectively in 21% (19/92) and 2,5% (2/77) (OR=0,11, p<0,0004). Similarly, HLA-A*0301 was found less frequently in diabetic children 9% (7/77) in comparison to control group 26% (24/92) (OR=0,3, p<0,01). Our results were confirmed in fenotyping analysis, where for HLA A*25 fenotype OR=15 (p<0,001), for HLA-A*0101 and HLA-A*0301 OR=0,1 (p<0,0003) and OR=0,27 (p<0,004) respectively, was obtained. There were an association between C-peptide level and the presence of HLA-A alleles. As the low level of C-peptide we accepted the level below 0,28 pmol/ml. Lower levels of C-peptide were found more frequently in diabetic patients with HLA-A*02 (OR=4,08, p<0,002). In patients with HLA-A*26 allel, we observed the level of C-peptide above 0,28 pmol/ml with higher frequency (OR=0,08, p<0,007). **Conclusions:** According with obtained results there is a significant correlation between HLA-A gene and the level of C-peptide in patients with type 1 diabetes mellitus at the onset. Explanation of this phenomenon requires further investigations.

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MAJOR ROLE FOR CTLA-4 GENE IN DR4-POSITIVE TYPE 1 DIABETIC PATIENTS AND IN DR3 NEGATIVE GRAVES' DISEASE PATIENTS

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Type 1 diabetes and Graves' disease are two autoimmune diseases associated with both HLA and CTLA-4 susceptibility genes. It is unknown whether these two genes interact in disease determination. **Aims:** In the present study we investigated HLA DR genes and CTLA-4 polymorphism in patients affected by Type 1 diabetes and Graves' disease. **Materials and Methods:** HLA class II alleles and the CTLA-4 exon 1 position 49 A/G dymorphism were typed in a population of continental Italians including 120 patients with type 1 diabetes, 102 patients affected by Graves' disease and in 112 healthy controls. HLA DR was performed by heteroduplex analysis, the CTLA-4 exon 1 position 49 A/G dymorphism was typed using restriction fragment length polymorphism after a specific PCR. **Results:** As expected, HLA DR3 and/or DR4 were significantly associated with Type 1 diabetes (p<0.0001, OR=3.9) whereas in patients with Graves' disease a weak association was observed with HLA DR3 (p=0.05). The CTLA-4 G phenotype was significantly associated with Type 1 diabetic patients positive for DR4 compared to non-DR4 patients (p<0.01, OR=3). On the contrary, in patients with Graves' disease the CTLA-4 G allele was significantly associated in non DR3 patients compared to controls (p=0.02). **Conclusions:** The association DR4-CTLA-4 G in Type 1 diabetes and of DR 3-CTLA-4 G in Graves' disease suggests a different interaction between these two major genes in the susceptibility to Type 1 diabetes and Graves' disease that can correspond to different clinical phenotypes.

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HYPERGLYCEMIA OF BB/OK MOTHERS INFLUENCES THE DIABETES FREQUENCY AND GENOTYPE OF CROSS OFFSPRING

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Aim: It is well-known that the risk to develop diabetes in offspring of type 1 diabetic fathers is 2-3 times higher than in those of diabetic mothers. This fact is commonly discussed in the sense that the diabetic state of mothers most probably protects their offspring from diabetes development. There are several speculations to explain this phenomenon, but the actual cause is unknown until now. Because the diabetes frequency in 61 *Iddm1* and *Iddm2* homozygous [(BB/OK x SHR)F1 x BB/OK] first backcross (BC1) offspring descending by backcrossing onto diabetic BB/OK mothers was significantly lower than onto diabetic BB/OK fathers (7.1 vs. 12.8%, p<.05), we analysed retrospectively this BC1 hybrids for the diabetes frequency and allele distribution at 114 microsatellite loci in dependence on their mothers age at onset of diabetes and age at delivery, litter size at birth and weaning, litter interval and glycemic state of mothers during pregnancy.

Results: From the traits studied, the glycemia of mothers being 256 ± 125 mg% in diabetic BB/OK mothers ranging from 140 to 480 mg% and 108 ± 10 mg% in non-diabetic F1 mothers (backcrossed onto diabetic BB/OK fathers) was the only factor influencing the diabetes frequency and allele distribution in offspring. The diabetes frequency in offspring of mothers with blood glucose of < 200 mg% during pregnancy was comparable between BB/OK and F1 mothers (41 vs. 46%), but was significantly reduced in offspring of BB/OK mothers with blood glucose > 200 mg% (8%, p<.05). In addition, the allele distribution in offspring was disturbed in dependence on glycemia of mothers. Increasing blood glucose values of mothers during pregnancy favoured heterozygosity at four loci on chromosome 1 in diabetic and non-diabetic offspring, particularly at locus *D1Mit14* (p=.0007) located close to insulin gene.

Conclusion: These findings clearly demonstrate the impact of hyperglycemia of mothers during pregnancy not only on diabetes frequency but also on the survival of genotype of offspring favouring heterozygosity.

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EARLY SOCIAL MIXING AT DAY CARE PROTECTS AGAINST CHILDHOOD DIABETES

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Aims: Evidence from animal models and epidemiological studies suggests the risk of developing Type 1 diabetes can be increased by the absence of early life exposure to pathogens. We tested this 'hygiene hypothesis' by investigating levels of social mixing and infections in the first year of life and the risk of developing childhood Type 1 diabetes.

Materials and Methods: In a population-based case control study in Yorkshire, UK we personally interviewed mothers of 220 children with Type 1 diabetes (0-15 years) and 433 age and sex matched controls. Social mixing, including attendance at day-care settings and infections occurring under 1 year of age were measures of exposure. Adjusted odds ratios were derived using conditional logistic regression.

Results: Frequency of attendance at day-care during the 1st year of life was inversely associated with the development of diabetes under 16 years of age (odds ratio 0.71, 95% confidence interval 0.51-1.00, P= 0.05), a finding not explained by mother's age, level of education or maternal diabetes. Increasing numbers of other children at day-care and number of sessions attended were significantly associated with increasing protection from diabetes. This reduced risk was strongest in children diagnosed under 4 years.

Conclusions: Social mixing through attendance at day-care in early infancy appears to confer protection against the development of childhood diabetes. This may be mediated through exposure to infectious agent(s) as a significant dose-response effect was evident with increasing numbers of 'contacts'. Our findings suggest early infectious exposure may play an important role in the development of immunoregulatory mechanisms which protect against diabetes.

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DOSE-RESPONSE RELATIONSHIP BETWEEN DURATION OF BREAST FEEDING AND AGE AT INTRODUCTION OF FORMULA BOTTLE FEEDING AND RISK FOR TYPE 1 DIABETES MELLITUS

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Aims: Type 1 diabetes results from a progressive autoimmune process associated with a destruction of the insulin-producing beta-cells of the pancreatic islets. Both genetic and environmental factors are thought to contribute to the development of the disease. During 1992-95 a nation-wide population-based case-control study was performed in Germany focusing on early risk factors for Type 1 diabetes in children under 5 years of age.

Methods: Altogether data from 767 incident cases (71.5% of eligible) and 1886 controls (43,8% of eligible), matched for age, sex, and place of residence, were analysed. Information on diet in early childhood, perinatal and socio-economic factors, and family history of Type 1 diabetes were collected using a mailed questionaire. Data were analysed by multivariate conditional logistic regression.

Results: A long duration of breast feeding was associated with a decreased risk for Type 1 diabetes. Compared to no breast feeding at all or a short duration of breast feeding of less than 2 weeks, odds ratios (95%-CI) for breast feeding periods of 2-6, 7-21, and ≥ 22 weeks were 0.93 (0.69-1.24), 0.85 (0.64-1.14), and 0.68 (0.52-0.88), respectively, clearly indicating a dose-response relationship (test for trend: p = 0.002). Likewise, a late beginning of formula bottle feeding significantly reduced the risk of Type 1 diabetes in a dose-response relationship (test for trend: p = 0.005). Compared to an introduction of formula within the first 2 weeks of life, odds ratios (95%-CI) for a beginning of bottle feeding at the age of 2-6, 7-21 or ≥ 22 weeks were 1.07 (0.80-1.42), 0.81 (0.62-1.07) 0.73 (0.56-0.94), respectively. Presented associations are adjusted for possible confounders (family history of Type 1 diabetes, recent cow's milk consumption, maternal age, social status, number of children in the family, and removal within the past 2 years).

Conclusions: This large nation-wide population-based case-control study showed a protective effect of a long duration of breast feeding and a late exposure with cow's milk. The finding of a dose-response relationship between these exposures and the risk of Type 1 diabetes indicates the observed associations to be causal.

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ATOPIC DISEASES, INFECTIONS, AND VACCINATIONS AND RISK FOR TYPE 1 DIABETES MELLITUS IN CHILDHOOD

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Aims: Type 1 diabetes results from a progressive autoimmune process associated with a destruction of the insulin-producing beta-cells of the pancreatic islets. Both genetic and environmental factors are thought to contribute to the development of the disease. During 1992-95 a nation-wide population-based case-control study was performed in Germany focusing on environmental exposures and the risk for Type 1 diabetes in children under 5 years of age.

Methods: Altogether data from 767 incident cases (71.5% of eligible) and 1886 controls (43,8% of eligible), individually matched for age, sex, and place of residence, were analysed. Information on atopic diseases, infections, antibiotic therapies and vaccinations were collected using a mailed questionaire. Data were analysed by univariate conditional logistic regression.

Results: Atopic eczema was significantly associated with a decreased risk for Type 1 diabetes (p = 0.003). The odds ratio (95%-CI) was 0.68 (0.52-0.88). Allergic rhinitis and asthma did not affect the diabetes risk. Varicella infection significantly reduced the risk for Type 1 diabetes (odds ratio: 0.68 (0.55-0.84), p < 0.001), while other common childhood infections (measles, mumps, rubella, pertussis, scarlet fever, recent unspecific infections) showed no association with diabetes risk. Recent antibiotic therapies, as proxy for severe infections, significantly increased the risk for Type 1 diabetes (p =0.041). The odds ratio for ≥5 antibiotic therapies in the past year compared to none was 1.68 (1.13-2.48). Completed vaccinations (≥3) against polio, diphteria/pertussis/tetanus and Haemophilus influenzae b were associated with a decreased risk for Type 1 diabetes. The odds ratios were 0.71 (0.55-0.92), 0.68 (0.51-0.91), and 0.54 (0.40-0.73), respectively. In tendency, a protective effect of a measles/mumps/rubella vaccination was observed (odds ratio: 0.79 (0.61-1.01), p = 0.067), while a BCG vaccination did not affect the diabetes risk.

Conclusions: The findings of this large nation-wide population-based case-control study, although not adjusted for possible confounders, indicate that atopic conditions and vaccinations, in particular the Haemophilus influenzae b vaccination, may be protective against the development of Type 1 diabetes.

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Vascular Dysfunction: In Vivo Studies

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TRANSCAPILLARY ESCAPE RATE OF ALBUMIN IS INCREASED AND RELATED TO HEMODYNAMIC CHANGES IN NORMOALBUMINURIC TYPE 1 DIABETIC PATIENTS

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Aims: An increase in urinary albumin excretion (UAE) in type 1 diabetic patients might reflect changes in vascular permeability and/or local hemodynamic factors. Indeed, transcapillary escape of albumin (TERalb), a measure of systemic capillary efflux, is increased in diabetic patients, even in those with a modest increase of albuminuria. Normoalbuminuric type 1 diabetes is characterised by an increase in capillary flow and glomerular hyperfiltration. We hypothesised that these hemodynamic changes contribute to an elevated transcapillary escape of albumin, even in the phase preceding microalbuminuria. **Methods** We calculated TERalb from the disappearance curve of ^{125}I-albumin in 39 normoalbuminuric (UAE <10 µg/min) type 1 diabetic patients and 46 healthy controls. Renal and systemic hemodynamics were measured by standard techniques. Forearm blood flow (FBF) was measured by plethysmography. Endothelial function was assessed by intra-arterial infusion of acetylcholine. The structural integrity of the vessel wall was determined by the post-occlusive reactive hyperemia test. **Results** TERalb was increased in diabetic patients (5.53±0.40 versus 4.39±0.21 %/hr, p=0.01). Patients were divided in tertiles with respect to their TERalb. Between the groups there were no differences in UAE, blood pressure, metabolic parameters, endothelial function or maximal vasodilatation after occlusion. However, filtration fraction and FBF were significantly increased in the group of diabetic patients with the highest levels of TERalb. Overall, in diabetic patients FBF was significantly correlated with TERalb (Pearsson r=0.51, p<0.01). **Conclusions** Transcapillary escape of albumin is increased in normoalbuminuric type 1 diabetic patients. In these patients with an increased capillary permeability there is no evidence of endothelial dysfunction or vessel wall damage. However, both forearm blood flow and filtration fraction are increased. Therefore, the increased vascular permeability in the early phase of type 1 diabetes is associated with general hemodynamic alterations. Notably, such an increase in vascular permeability is not necessarily reflected by abnormal urinary albumin excretion.

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RESISTANCE VESSEL FUNCTION AND STRUCTURE IN NORMOTENSIVE PATIENTS WITH TYPE 1 DIABETES.

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Aims: The pathogenesis of the microvascular complications of diabetes is poorly understood. Resistance vessels determine capillary blood flow and thereby may regulate the development of microvascular complications in diabetes. **Materials and Methods:** We have studied 24 normotensive (BP-128.4 ± 16.8/ 76.6 ± 10.2), long duration (24.9 ± 11.0 yr.) type 1 diabetic patients with varying degrees of microvascular complications, and 5 control subjects. Resistance vessels were dissected from gluteal fat biopsies and their function and structure assessed in a pressure myograph. **Results:** The maximal relaxation to acetylcholine (endothelium dependent) before (87.3 ± 4.3 v controls 96.1 ± 2.7), and after blocking with LNMMA (63.8 ± 5.1 v controls 66.9 ± 8.7) and the derived contribution of NO (26.9 % v controls 30.3 %) did not differ significantly. Ach Max correlated significantly with HbA1c (r=0.61, p<0.002), glucose concentration at biopsy (r=0.69, p<0.0001), total cholesterol (r=0.40, p<0.06) and HDL (r=0.39, p<0.06). Maximal relaxation to sodium nitroprusside (endothelium independent) was greater in diabetic patients (79.2 ± 4.6) v control subjects (68.2 ± 12.3). Noradrenaline induced vasoconstriction did not differ between diabetic patients (75.7 ± 2.8) v controls (75.4 ± 4.2). However, Angiotensin II induced maximal vasoconstriction (35.1 ± 4.4 v controls 18.6 ± 8.9) and the EC50 (5.2 ± 0.7 v controls 3.1 ± 1.4) was increased in diabetic patients v controls. The wall/lumen ratio (0.26 ± 0.01 v 0.37 ± 0.05, p<0.04) and the maximal vascular dilatation in response to increased intraluminal pressure (200mmHg) (66.9 + 4.3 v 82.2 + 17.5) was decreased in diabetic patients. **Conclusions:** In normotensive long-duration patients with Type 1 diabetes, resistance vessel endothelium dependent relaxation is unaltered whilst endothelium independent relaxation is increased. Constriction and sensitivity to Angiotensin II is enhanced and may explain the enhanced benefits of ACE inhibition in diabetes. Resting dilatation is consistent with the haemodynamic hypothesis of capillary damage. Impaired dilatation with increasing intra-luminal pressure may further enhance damage via shear stress.

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IN VIVO ENDOTHELIAL DYSFUNCTION CHARACTERIZES PATIENTS WITH IMPAIRED FASTING GLUCOSE (IFG)

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Aims. ADA has recently defined an intermediary group of subjects entitled 'IFG', whose glucose levels do not meet the criteria of diabetes but are too high to be considered normal (Normal). We determined whether endothelial dysfunction characterizes subjects with IFG.

Materials and Methods. *In vivo* vasodilatory responses to intra-arterial infusions of endothelium-dependent (acetylcholine, ACh) and -independent (sodium nitroprusside, SNP) vasoactive agents were determined in 29 non-diabetic males, whose glucose tolerance was classified according to ADA criteria:

	n	age (yrs)	BMI (kg/m^2)	MAP (mmHg)	fP-Gluc (mmol/l)	Insulin (mU/l)	LDL chol (mmol/l)	TG (mmol/l)
Normal	12	60±1	26.2±0.6	108±2	5.6±0.1***	7±1	3.8±0.2	1.1±0.1
IFG	17	63±1	26.5±0.8	101±3	6.5±0.1	9±2	3.5±0.2	1.2±0.1

Data are shown as mean ±SEM. *** p<0.001 for Normal vs IFG

Results. The blood flow responses to both low and high doses of ACh were significantly and by 46-31 % blunted in the IFG (5.9±0.7 and 9.1±1.2 ml/dl·min, low and high dose) compared to the Normal group (10.9±1.3 and 13.2±1.5 ml/dl·min, respectively, p<0.01 and p<0.05). In contrast, blood flow responses to both low and high doses of SNP in the IFG group (7.8±0.5 and 11.6±1.2 ml/dl·min) were comparable to those in the Normal group (9.0±0.9 and 12.3±1.3 ml/dl·min). The ratio of endothelium-dependent to -independent blood flow (ACh/SNP) was 40 % lower in the IFG (0.75±0.1) than the Normal group (1.24±0.1, p<0.001). Fasting plasma glucose was also significantly inversely correlated with both the blood flow response to ACh (r=-0.48, p<0.01) and to ACh/SNP (r=-0.42, p<0.05).

Conclusions. In summary, in vivo endothelial dysfunction characterizes individuals with impaired fasting glucose. These data are consistent with the idea that vascular dysfunction precedes overt hyperglycemia in type 2 diabetes.

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EXPRESSION OF ENDOTHELIAL NITRIC OXIDE SYNTASE (eNOS) IN IMPAIRED GLUCOSE TOLERANCE (IGT) AND TYPE 2 DIABETES.

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Aims. We have previously shown that vascular reactivity in the micro- and macro-circulation is impaired in individuals either with type 2 diabetes or at risk for it. We have now examined the expression of eNOS in the microcirculation in the same conditions. **Methods.** Full thickness skin punch biopsies were taken from the forearm, same area where functional microcirculation measurements were taken, of 11 healthy controls [4 (36%) males (M), age 45 ± 7 yrs (mean ± sd)], 11 healthy subjects with history of parental type 2 DM [2 (18%) M, age 50 ±7], 12 subjects with IGT [8 (67%) M, age 53 ± 12] and 8 patients with uncomplicated type 2 DM [6 (75%) M, age 56 ± 10, duration of DM 6 ± 5 yrs]. Biopsies were immunostained with antiserum to human eNOS, and von Willebrand factor which is an anatomical marker of the endothelium. The staining intensity of the skin microvessels was evaluated by a pathologist, who was blinded to the glycemic status of each subject. **Results** Staining for von Willebrand factor was present and of the same intensity in all four groups. In contrast, the staining for eNOS was absent in a higher percentage of diabetic patients [6 (75%)] and subjects with IGT [8 (67%)] compared to relatives [2 (18%)] and controls [4 (36%), (p <0.05). Binary logistic regression analysis showed a significant association with sex (OR 0.24, p<0.05), BMI (OR 0.87, p<0.05) and 2 hour glucose after OGTT, (OR 0.97, p<0.02). Multivariate logistic regression including the above factors showed that statistical significance hold only for the 2h glucose value (OR 0.97, p<0.05). Staining with antiserum to PPAR γ showed intense staining in fat cells and eccrine glands but minimal or absent staining of the endothelium in the microvessels. These findings are consistent with our previous study in which we have demonstrated that endothelium dependent and independent vasodilation in the micro- and macrocirculation is impaired in subjects at risk of developing type 2 diabetes. **Conclusions.** The expression of e-NOS is reduced in subjects with impaired glucose metabolism and may be a significant contributing factor for the reduced vascular reactivity observed in this condition.

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IMPAIRED VASCULAR REACTIVITY AND ENDOTHELIAL ACTIVATION IN TYPE 2 DIABETES WITH OR WITHOUT MICROALBUMINURIA.

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Aims. Microalbuminuria is a marker of cardiovascular risk in diabetic individuals and has been associated with endothelial dysfunction. To further assess this situation, we compared vascular reactivity and plasma levels of various markers of endothelial activation in type 2 diabetic patients with and without microalbuminuria and healthy controls. **Methods.** We employed laser Doppler imaging scanning to measure vasodilatation in the forearm skin in response to iontophoresis of 1% acetylcholine (Ach, endothelium-dependent) and 1% sodium nitroprusside (SNP, endothelium-independent) in 3 groups: 20 healthy normoglycemic subjects [C group, 10M/10F, age 53 ± 6 (mean ± sd) years], 45 type 2 DM patients without microalbuminuria (D-, 14M/31F, age 54 ± 10, DM duration 5.1±5.8 years) and in 14 type 2 DM patients with microalbuminuria (D+, 5 M/9F, age 55 ± 9, DM duration 5.4 ± 3.7 yrs, alb/cr ratio 30-300 μg/m). Plasma levels of sICAM, sVCAM and vWF were measured in all groups. **Results** FPG was higher in D+ compared to D- (226 ± 61 mg//dl vs 147± 30 p<0.001), as well as HbA1c (9.4± 1.7% vs 7.4± 0.8), T-cho' (233± 33 mg/dl vs 205± 34), LDL-C (156 mg/dl ± 39 vs 124 ± 32) and Tg (213 ± 103 mg/dl vs 197 ± 95). There was no difference in BMI, BP, insulin and HDL-C levels between D+ and D-. All metabolic variables were higher in D+ and D- compared to C. A similar reduction in vascular reactivity to Ach was seen in D+(74 ± 41 % increase over baseline) and D- (75 ± 43) compared to C (110 ± 44)(p< 0.05). The same pattern was observed in the response to SNP (D+ 73 ± 28, D- 73 ± 35, C 122 ± 41, p< 0.001). sICAM was higher in D+ (300 ± 89 ng/ml) and D-(305 ± 120) compared to C (213 ± 58)(p<0.01). sVCAM was also similarly higher in D+ (795 ± 297 ng/ml) and D-(839 ± 238) compared to C (654 ± 173)(p<0.01). However, vWF was higher in D+ (154 ± 35%) compared to D- (110 ± 34) and C (111 ± 39)(p<0.05). **Conclusions.** Multiple markers of endothelial dysfunction are present in normoalbuminuric individuals with type 2 diabetes mellitus. The pathogenic process of vasculopathy in type 2 diabetes occurs early and seems to be operative before the development of microalbuminuria.

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INSULIN SENSITIVITY AND ENDOTHELIAL FUNCTION IN "CARDIAC SYNDROME X" AND "METABOLIC SYNDROME X"

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Aim: To characterize the endothelial and metabolic alterations of patients with angina and angiographically normal coronary arteries ("cardiac syndrome X") compared to subjects with Insulin Resistance Syndrome ("metabolic syndrome X") and normal subjects. **Materials and Methods:** 35 subjects were studied: 13 patients with "cardiac syndrome X" (group 1); 9 subjects with "metabolic syndrome X" (group 2) and 13 normal controls (group 3). All subjects received an acute intravenous bolus of insulin (0.1 U/kg) combined with a euglycemic clamp and forearm indirect calorimetry. Endothelin-1 (ET-1) levels, nitrite/nitrate (NOx) levels, end products of nitric oxide metabolism, glucose infusion rates (GIR), an index of insulin sensitivity and their incremental areas (ΔAUCs) were measured during this period. **Results:** Basal ET-1 levels were higher in groups 1 and 2 than in group 3 (8.19±0.46 and 6.97±0.88 vs 3.67±0.99 pg/ml; p<0.01) while basal NOx levels were significantly higher in group 2 than in groups 1 and 3 (36.5±4.0 vs 24.2±3.3 and 26.8±3.2 μmol/l, p<0.05). After insulin administration, the ΔAUCs of NOx (p<0.05) were lower in group 1 than in groups 2 and 3 and the ΔAUCs of ET-1 were lower in group 1 than in group 3. GIR was significantly lower in groups 1 and 2 than in group 3 (1242.2±126.7 and 651.4±66.1 vs 2143.6±178.7 μM/kg/min, p<0.05). Interestingly, GIR was significantly higher in group 1 than group 2 (p<0.05). A positive correlation was found between the ΔAUCs of nitric oxide and the AUCs of GIR. **Conclusions:** "cardiac syndrome X" and "metabolic syndrome X" show different degree of insulin resistance. Moreover, even if high ET-1 levels are present in both groups, blunted nitric oxide and endothelin responsiveness to insulin seems a typical feature of patients with "cardiac syndrome X".

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FREE FATTY ACIDS ALTER ENDOTHELIUM-DEPENDENT VASODILATION IRRESPECTIVELY OF CHAIN LENGTH AND PROSTAGLANDIN PRODUCTION.

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Aims. We investigate whether the chain length of free fatty acids (FFA) and their ability to induce prostaglandin (PG) synthesis might affect their inhibitory action on endothelium-dependent vasodilation (EDV). Materials and Methods. Seven of 14 controls (C) were randomly allocated to 2 of these 4 studies: 1. Baseline condition; 2. Infusion of long chain triglyceride (LCT) emulsion (2 ml/min + Heparin 40 $U.kg^{-1}.h^{-1}$); 3. Infusion of emulsion with 56% medium chain triglycerides (MCT) and 44% LCT (2 ml/min + Heparin 40 $U.kg^{-1}.h^{-1}$); 4. Infusion of LCT + Heparin preceded by an i.v. bolus of aspirine lysine (ASA) 900 mg; 5. After an i.v. bolus of ASA 900 mg. EDV in response to intraarterial acetylcholine (Ach) and endothelium independent vasodilation in response to intraarterial nitroprusside (SNP) were assessed by plethysmography. Results. Both LCT and MCT increased basal forearm blood flow (FBF) from 1.58±0.35 $ml.min^{-1}.100$ ml $tissue^{-1}$ to 2.60±0.76 and to 2.28±0.56 (both $p<0.05$). The same increase was also observed for LCT+ heparin but not after ASA alone. The increase in FBF during Ach was depressed both during LCT (252±34% of the ratio infused:control arm at maximal Ach dose) and MCT (255±41%) as compared to baseline (436±44%, both $p<0.05$). The response to Ach was depressed during LCT + ASA, whereas it was similar to baseline with ASA alone. No differences were observed in response to SNP. Conclusions. 1. LCT and MCT increase FBF independently from PG synthesis. 2. The inhibitory effect of FFA on EDV takes place irrespectively of their chain length. 3. PG synthesis does not interfere with the inhibitory action of FFA on EDV. 4. ASA does not acutely affect EDV. Elevated FFA concentrations, such those observed in conditions of insulin resistance, may negatively affect EDV, thus contributing to the development and progression of atherosclerotic complications.

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MICROVASCULAR DYSFUNCTION AND MYOCARDIAL CONTRACTILITY IN THE JCR:LA-CORPULENT RAT

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Aims: The cp/cp genotype of the JCR:LA rat develops a fully expressed metabolic syndrome, featuring obesity, pronounced insulin resistance, dysglycemia and hyperlipidemia. With aging, in addition to macrovascular disease, these animals develop ischemic myocardial lesions based on small vessel disease. In contrast to findings in large conductance arteries, no data on endothelial function in coronary resistance vessels of the JCR:LA rat exist. **Materials and Methods:** Endothelial and smooth muscle function of coronary resistance vessels was assessed in JCR:LA cp/cp rats aged 18 weeks and compared to that in lean littermates (cp/+ and +/+). Hearts were perfused retrogradely at 15 ml/min per g which resulted in a coronary perfusion pressure (CPP) of 133±1 mm Hg (baseline). The effects of bradykinin (0.1-1000 nmol/l) or spermine/NO (100 µmol/l) on CPP and left-ventricular developed pressure (LVDP) were determined in absence and presence of either L^G-nitro-L-arginine (L-NNA; 0.2 mmol/l) or indomethacin (10 µmol/l). **Results:** Bradykinin reduced CPP down to 50±2 mm Hg (-62 %) in +/+ rats, but only to 73±2 mm Hg (-45 %) in cp/cp rats (n=8, $P<0.05$). The respective EC_{50} values were 0.45 and 1.0 nmol/l. In presence of L-NNA, relaxation was attenuated to the same degree in both groups (-27 %; EC_{50}: ~6 nmol/l), whereas indomethacin was ineffective. Spermine/NO equally reduced CPP in both groups (-58 %). Finally, baseline LVDP was not different between groups. **Conclusions:** These findings suggest that there is a specific impairment of nitric oxide mediated endothelium-dependent relaxation of the coronary resistance vessels in the corpulent male rat that is not associated with impaired baseline myocardial contractility.

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Insulin Secretion and Development of Type 2 Diabetes

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INSULIN SECRETION AND SENSITIVITY IN RELATION TO GLUCOSE TOLERANCE- RESULTS FROM THE BOTNIA STUDY

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The aim of the study was to assess insulin secretion and insulin sensitivity in subjects with varying degrees of glucose tolerance, particularly to compare impaired fasting glucose (IFG , FPG: 6.1-6.9 mmol/l) and impaired glucose tolerance (IGT, 2 hr glucose 7.8-11 mmol/l). To address this question we studied 7087 individuals in the Botnia study by oral glucose tolerance test (OGTT), and a subset of them with intravenous glucose tolerance test (IVGTT, n=647) and euglycemic clamp (n=291). There was a poor concordance between IFG and IGT; only 36% (319/879) of subjects with IFG had IGT, whereas of 62% of IGT subjects (525/844) did not have IFG. Compared to subjects with normal glucose tolerance (NGT, n=3274), IFG subjects (n=560) were more insulin resistant as shown by higher HOMA-IR values (2.66± 0.45 vs 1.72 ± 0.17, $p<0.05$), greater insulin area during OGTT(5336 ± 142 vs 4449± 59, $p<0.05$), higher waist to hip ratio ($p<0.05$) , triglycerides (1.42 ± 0.05vs 1.21 ± 0.02 mmol/l, $p<0.05$), total cholesterol (5.75 ± 0.04 vs 5.43 ± 0.02mmol/l, $p<0.05$) and lower HDL cholesterol (1.31 ± 0.01 vs 1.37 ± 0.006mmol/l, $p<0.05$) concentrations. IGT (N=525) subjects differed from IFG subjects by virtue of impaired insulin secretion. The early insulin /glucose response at 30 min of the OGTT (13.8 ±1.7 vs 21.7 ± 1.7, $p<0.05$) and the first phase insulin response during IVGTT adjusted for insulin sensitivity (149 ± 37 vs 270 ± 15mU/l, $p < 0.05$) were lower in IGT vs IFG and /or NGT, although this was not reflected by absolute insulin values. A progressive decline in insulin mediated glucose uptake was observed when moving from NGT to diabetes (FPG 7.0-7.8 mmol/l) (from 6.91 to 4.94 $mg^{-1}kg^{-1}min^{-1}$, p for trend <0.05). However, the slope of decline in FPI was markedly steeper(from 285.7 to 74.1 mU/l). In conclusion, these findings suggest that IFG is characterised by basal insulin resistance and other features of metabolic syndrome while IGT subjects show impaired insulin secretion in relation to glucose concentrations and insulin sensitivity. An absolute decompensation of β-cell function characterizes the transition from IGT to mild DM.

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IMPAIRED INSULIN SECRETION AND SENSITIVITY IN MODY4.

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Diabetes due to a heterozygous mutation of the homeodomain transcription factor Insulin Promotor Factor-1 (IPF-1) is called MODY4. Aims: To quantitate islet cell response in MODY4. Materials and Methods: We studied 15 members of a family harboring Pro63fsdelC mutation in IPF-1. Seven members carry the IPF-1 mutation (NM; mean (X) age 47, X BMI 26.6) and 8 have a normal genotype (NN; X age 37, X BMI 19.7). Glucose (GL) clamps were performed at 5 hyperglycemic steps. Each step lasted 1 hr. Basal GL (NM=9.2, NN=5.9 mmol/l) levels were raised by 5.6 mmol/l initially and by 2.8 for each of the subsequent 4 hrs. During the last ½ hr of the 5th step, GLP-1 was also infused (1.5 $pmol \cdot kg^{-1} \cdot min^{-1}$). Results: Plasma GLP-1 levels were ~175 pmol/l during GLP-1 infusion. Basal IRI levels for the NM and NN groups were 72 and 105 pmol/l. First phase response occurred only in the NN group. The NM had markedly attenuated IRI responses to GL alone compared to NN. The 240-270 min levels averaged 596±238 and 4098±908 pmol/l. GLP-1 potentiated IRI secretion in both NM and NN groups, but the NM group has a 7-fold lesser response to GLP-1 (1481±691 vs. 9998±1703). C-peptide responses to GL followed the pattern of IRI responses. In both groups glucagon levels fell during each glycemic plateau and further reduction occurred during GLP-1 infusion. Basal NEFA levels were 0.78 mmol/l in the NM group and dropped to 0.18 by the end of study. Corresponding values for the NN group were 0.52 and 0.22. Sigmoidal dose response curves of GL clearance ($ml \cdot kg^{-1} \cdot min^{-1}$) vs. IRI levels were generated for NM (R^2=0.92) and NN (R^2=0.97). Both a left shift and a lower maximal response in the NM group compared to the NN group occurred. The ED_{50} values for IRI in the NM and NN groups were 348 and 874 pmol/l. Conclusions: IPF-1 mutation is associated with severe impairment of β-cell sensitivity to GL with an increase in peripheral tissue sensitivity to IRI. IPF-1 haploinsufficiency causes diabetes.

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INSULIN SENSITIVITY, GLUCOSE EFFECTIVENESS AND β-CELL SENSITIVITY IN DIABETIC AND NON DIABETIC SUBJECTS CARRYING THE R272H HNF-1α GENE MUTATION (MODY3).

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One form of Maturity Onset Diabetes of the Young (MODY) results from mutations in the gene encoding hepatocyte nuclear factor-1alpha (HNF-1alpha) located on chromosome 12 in band q24 (MODY3), involved in tissue-specific regulation of liver genes but also expressed in pancreatic islets. **Aim:** to study insulin secretion and sensitivity in MODY3 subjects. **Materials and Methods:** we performed an insulin modified frequently sampled iv glucose tolerance test (FSIVGTT) (dose= 0.3 gr/Kg 50% dextrose at basal time plus 0.05 U/Kg regular insulin at 20th min) in five adult diabetic and two pubertal nondiabetic (PND) members of a large pedigree carrying the R272H HNF-1α gene mutation. Insulin sensitivity (Si), glucose effectiveness (SG) and the sensitivity to glucose of the first (ϕ1) and second (ϕ2) phase of pre-hepatic β-cell insulin secretion were studied by analyzing the glucose, insulin and C-peptide data with the minimal model method. **Results:**

Diabetic phenotype (n)	ϕ1 (10^9)	ϕ2 ($min^{-1}x10^9$)	Si ($min^{-1}mcU^{-1}ml10^{-4}$)	SG ($min^{-1}x10^2$)
	M±SD	M±SD	M±SD	M±SD
IDDM (2)	7,5±6,5	0,00	1,78±0,1	1,51±0,01
NIDDM (3)	204±82.2	2.75±0.2	4.3±1.7	2.1±0.8
adult controls (10)	162±18	8.7±0.8	5.5±1.5	2.5±0.7
nondiabetic patients(2)	368,5±125	18,8±3,2	3,83 ±1,5	2,7±0,6
pubertal controls (4)	182±56	14.4±5.2	4.3±1.7	2.1±0.9

Conclusions: NIDDM patients showed a significant decrease only of ϕ2($p=0.0001$). Si was significantly lower only in IDDM patients ($p=0.007$).The PND subjects showed an hyperisulinemia with insulin resistance similar to their matched controls.The study of a greater number of genetically affected members of this MODY3 family is planned to better evaluate the role of the pancreatic insulin secretion and the Si defects in the development of this glucose metabolism disorder.

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INSULIN SECRETION IN GLUCOSE TOLERANT OFFSPRING CORRELATES WITH AGE OF ONSET OF TYPE 2 DIABETES IN THE PARENTS.

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Aim: Obesity, insulin resistance and a low acute insulin response (AIR) to glucose predict the development of type 2 diabetes in Pima Indians in whom segregation analyses suggest the presence of a major gene affecting age of onset of diabetes. To determine the mechanisms that increase the risk for diabetes in the offspring of Pima Indians with early onset diabetes in the parents, we conducted 2 studies. **Materials and Methods**: 1) The relation of obesity and insulin secretion (25-g intravenous glucose test) and action (hyperinsulinemic glucose clamp) to age of onset of diabetes in the parents was examined in 104 Pima Indians with normal glucose tolerance (age 22±1y, body fat 33±1%, mean±SE) and whose mothers were not diabetic before pregnancy. 2) Insulin secretion rates (deconvolution of C-peptide) during a stepped glucose infusion were measured in subjects whose mothers developed type 2 diabetes before age 35 (n=8) or after age 49 (n=15). **Results**: 1) AIR correlated with the age of onset of diabetes in the mother ($r=0.23$, $p<0.05$). In multiple regression analyses, the interaction between the age of onset of diabetes in the mother and in the father was a significant determinant of AIR ($p<0.05$). Insulin action and body fat were not correlated with the age of diabetes onset in the parents. 2) In response to stepped glucose infusions, the offspring of mothers with early onset diabetes had lower average insulin secretion rates compared to offspring of mothers with late diabetes onset: 439±86 vs 650±80 pmol/min ($p<0.01$). **Conclusion**: Factors inherited from parents with early onset diabetes impair insulin secretion to increase the risk for type 2 diabetes in Pima Indians.

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GLUCOSE PULSE INDUCTON MARKEDLY IMPROVES DETECTION OF A DISRUPTED OSCILLATORY INSULIN SECRETION IN TYPE 2 DIABETES.

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Intrapancreatic mechanisms are assumed to coordinate the high frequency pulsatile insulin release, but occurrence of small oscillations in glucose concentrations may contribute. In a diabetic animal model and in healthy humans we have previously demonstrated that tiny glucose excursions (~ 0.3 mM) are able to modify the rapid pulsatile insulin secretion. To gain further insight into beta-cell pathophysiology, we explored the ability of small repeated glucose infusions (6 mg/kg/ 1 min every 10 min) to influence rapid pulsatile insulin secretion in 9 mild type 2 diabetic individuals (fasting plasma glucose 9.3 ± 1.0 mM, HbA1c 8.0 ± 0.6 %) and 9 healthy subjects. All subjects were examined during saline and during glucose exposure and blood was collected every min for 90 min to establish robust serum insulin time-series which were evaluated employing three different and complementary algorithms, namely autocorrelation and spectral analyses and approximate entropy (ApEn). Without induction (saline) none of the algorithms was able to discriminate between diabetics and controls for a ten min periodicity (all $p>0.20$) During glucose administration autocorrelation coefficients (AC), spectral density peaks (SP) significantly increased ($p<0.001$) and ApEn decreased ($p=0.01$) as compared to saline in the controls, all indicating more regular insulin time-series. However, no difference was observed among the diabetics during the two conditions. Notably, glucose entrainment led to an almost complete separation of the insulin time-series between diabetics vs. controls (AC: 0.03 ± 0.01 vs. 0.65 ± 0.06; SP: 1.25 ± 0.22 vs. 8.76 ± 0.82; ApEn: 0.689 ± 0.010 vs. 0.513 ± 0.022; all $p<0.001$). The present study elucidates a novel dimension of the scenario of disrupted beta-cell function in type 2 diabetes mellitus and demonstrates that recurrent discrete glucose pulses improve the ability to detect abnormalities of rapid oscillatory insulin release. The method may turn out to be useful in unmasking subtle (prediabetic) defects in beta-cell sensitivity to glucose.

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GLUCOSE-INSULIN SECRETORY DOSE-RESPONSE CURVES DURING CONSTANT GLP-1 INFUSION AND PRETREATMENT WITH GLP-1.

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Glucagon-like peptide-1, a natural enteric peptide, has been shown to improve glucose induced insulin secretion. Using patch clamp techniques on isolated islets, it has been shown that only a subgroup of pancreatic ß-cells are sensitive to glucose and that pretreatment of cells with GLP-1 increases the number of glucose-competent ß-cells. Aims:, The aim of this study was to establish in normal volunteers the alterations in ß-cell responsiveness associated with a constant infusion of GLP-1 and a 60 minute pretreatment infusion of GLP-1. Materials and Methods:, 10 normal volunteers mean age 25.3±0.7 years, BMI 23.5±0.5 kg/m^2 were studied on 3 separate occasions using a graded glucose infusion protocol starting with an infusion rate of 1 mg/kg/min followed by 4, 8, 16 and 24 mg/kg/min (each infusion rate lasting for 40 minutes), in the presence of a saline, GLP-1 @ 0.4 pmol/kg/min, or pretreatment with GLP-1 @ 0.4 pmol/kg/min for 60 minutes. Blood samples were drawn at 10 min intervals for the measurement of glucose, C-peptide, insulin and glucagon. Insulin secretion rates (ISRs) were calculated by deconvolution of peripheral C-peptide levels using a two-compartment model utilizing mean kinetic parameters. Results:, From 5 to 9 mmol/l glucose, the relationship between glucose and ISR was linear. Constant GLP-1 infusion resulted in an increase in ISR from 5-9 mmol/l glucose from 253.7±33.8 pmol/min during saline infusion to 792.8±103.7 pmol/min ($P<0.0001$), with a shift in the dose-response curve to the left. Pretreatment with GLP-1 resulted in a positive response in 5 subjects with ISR increasing from 226.6±40.5 vs 322.4±23.2 pmol/min ($P<0.02$), and no response in 5 subjects 280.9±55.9 vs 258.1±48.3 pmol/min ($P=0.13$). Mean glucagon levels were not significantly reduced during the constant infusion of GLP-1, 63.1±6.1 vs 58.3±5.6 pg/ml ($P=0.5$). Conclusions:, In normal subjects constant infusion of GLP-1 resulted in a 216 % increase in ISR in the glucose range 5-9 mmol/l. Pretreatment with GLP-1 caused priming of ß-cell function in only 50 % of subjects with a 42% increase in ISR. Possible mechanisms for the priming effect of GLP-1 include increased insulin biosynthesis, increased GLUT2 expression, increased glucokinase mRNA and induction of ß-cells into a secretory mode

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MODIFIED HYPERGLYCAEMIC CLAMP DESIGNED TO DETECT DEFECTS IN GLUCOSE, INCRETIN AND ARGININE INDUCED INSULIN SECRETION

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Aims: Characterization of insulin secretion is essential for studying the genetic defects leading to type 2 diabetes. The classical tests for assessing beta cell function (IVGT, hyperglycaemic clamp) only provide information about glucose induced insulin secretion. To gain multifaceted information on islet function for use in subjects at genetic risk, we developed a hyperglycaemic clamp (HC) combined with a glucagon-like peptide 1 (GLP-1) infusion and an arginine bolus. The reproducibility of this clamp was tested. **Materials and Methods:** 7 lean, healthy volunteers (27 ± 2 years old) with normal glucose tolerance twice underwent a 200 minute HC (10 mmol/l) with administration of GLP-1 (1.5 pmol • kg body weight^{-1} • minute^{-1}) starting at 120 minutes and a 5 g arginine bolus at 180 minutes. We calculated glucose induced 1st (sum of 2.5, 5, 7.5, 10 min values) and 2nd phase (mean of 80, 100, 120 min values) insulin secretion, GLP-1-stimulated insulin secretion (mean of 160, 170, 180 min value, incretin phase) and an arginine stimulated insulin (ASI) and glucagon (ASG) secretion (sum of 182.5, 185, 187.5, 190 min values). **Results:** Insulin levels increased from 45±14 to 505±168 pmol/l during the first 120 minutes (HC). During GLP-1 infusion insulin increased to 5238±923 and after arginine to 7889±1367 pmol/l. The within subject coefficient of variation (CV) was 10.4±3.4% for the 1st phase and 11.0±2.6% for the 2nd phase of insulin secretion. The incretin phase showed a CV of 16.0± 5.6, whereas ASI and ASG showed CV's of 10.8±2.5 and 8.6±3.8, respectively. Incretin phase correlated with 2nd phase (r=0.82) and ASI with 1st phase (r=0.95) (both $p<0.05$). **Conclusions:** The insulin secretion parameters of the test are highly reproducible and show a high intraindividual correlation. It can thus be used to identify defective stimulation of insulin secretion by 3 different secretagogues during a single experimental session.

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Bivariate Frequency Analysis Of Glucose Oscillations And Pulsatile Insulin Secretion During The IVGTT For Type 2 Diabetic Patients And Controls

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During i.v. Glucose Tolerance Tests (IVGTT) not only insulin secretion but also glucose concentrations demonstrate oscillations. Bivariate Frequency Analysis allows the description of interdependence of such time series. *Patients* : 6 newly diagnosed male type 2 diabetic patients, 6 male controls. Age 55±9 (41±7), BMI 27,1±1,3 (24,8±1,0) HbA1c 7,1±0,8 (5,1±0.3). *Methods* : modified IVGTT (0,3 g glucose/kg i.v. in 30 sec. Insulin and glucose at –30, -10, -5, then simultaneously in periods of 1 minute from 0 to 30 min, 40,50,60,90,120,150,180 min. Insulin with double antibody Elisa, proinsulin free (Abbot), Glucose with GOD-method (Beckman analyzer). *Time series analysis:* Data were pooled, detrended by differences and seasonality removed by log transformation. The centered data passed a Tukey-Hamming window with a span of 31. *Results* : Univariate spectral density estimates for Insulin show maxima at a period of 3 and 5 min for controls and 2.5 and 5 min for type 2 (loss of density for 5 min). Maxima for glucose separate in controls at 2.5 min and for type 2 at 3 min. Cross amplitudes for glucose and insulin in controls show a maximum below 3 min with an estimated amplitude of 30×10^3. In type 2 there is one maximum in the same period length below 3 min,(amplitude 100×10^3) and an other at 4.5 min (amplitude 80×10^3).The phase spectrum of insulin and glucose for controls has a shift of 3 min at a period length of 2-2.5 min and a shift of 2.5 min at a period length of 2.5 – 3.5 min for type 2. *Conclusion:* There are clear dynamic oscillatory interdependences of the glucose and insulin system.The phase shift (time lag) between the insulin and glucose oscillation is about 3 min and not significantly different for controls and type 2. The pattern of dynamic interdependence is different for type 2 and controls, but the oscillatory strength of the dynamic interaction seems even to be more pronounced in type 2.

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Gestational Diabetes

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GESTATIONAL DIABETES MELLITUS IS ASSOCIATED WITH A TRP64ARG POLYMORPHISM OF THE β_3-ADRENERGIC RECEPTOR GENE

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A missense mutation of the β_3-adrenergic receptor gene (Trp64Arg) has been proposed as a potential modifying factor in the etiology of type 2 diabetes and the development of features of the insulin resistance syndrome; furthermore, this polymorphism has been associated with obesity and an increased capacity to gain weight.

The aim of our study was therefore to relate the Trp64Arg polymorphism of the β_3-adrenergic receptor gene to body weight, weight gain, glucose tolerance status and various metabolic variables during an oral glucose tolerance test (mean: 25th week of pregnancy) in a large number (n=179) of consecutive, caucasian, pregnant women.

Methods: The β_3-adrenergic receptor genotype was assessed using restriction fragment length polymorphism. Gestational diabetes was diagnosed if the 1h serum glucose value during the OGTT was ≥ 160 mg/dl (8.9 mmol/l).

Results: In women with mild gestational diabetes (n=70), the Typ64Arg genotype was more frequent compared to women with normal glucose tolerance (n=109) (25.7% vs. 11.0% p=0.01). Women with the Trp64Arg polymorphism showed an enhanced increase in body weight and BMI during pregnancy and increased post-load glucose, insulin and C-peptide values during the OGTT.

	Trp64Trp	Trp64Arg	p-value
N(%)	149(83.2)	30(16.8)	
Body weight (kg, initial)	65.1 ± 1.5	60.0 ± 1.9	n.s.
Δ Body weight (kg)	6.3 ± 0.4	8.6 ± 0.8	0.02
$Glucose_{60}$ (mmol/l)	7.48 ± 0.22	8.77 ± 0.46	0.02
$C\text{-}peptide_{60}$ (ng/ml)	9.6 ± 0.3	11.9 ± 0.4	0.02
$Insulin_{60}$ (pmol/l)	433.8 ± 21.0	505.2 ± 48.0	0.17

Conclusion: The present study extend current knowledge about the association of the Trp64Arg β_3-adrenergic receptor polymorphism with glucose tolerance to a pregnant population. The association with mild gestational diabetes mellitus suggests that the impact of the polymorphism may be clinically important during pregnancy.

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ELEVATED PLASMA LEPTIN IN GESTATIONAL DIABETES

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Aims: Leptin is supposed to play a role in body weight regulation by neuroendocrine mechanism, thereby causing a decrease in food intake and an increase in energy expenditure. The impact of pregnancy and glucose homeostasis on plasma leptin was investigated in 12 normal-weight women with gestational diabetes (GDM) and 10 pregnant women with normal glucose tolerance (NGT) matched for age and preconceptual BMI (age: 28.4±1.9 vs. 28.5±2.8 yrs, BMI: 24.7±1.7 vs. 23.9±1.2 kg/m^2). **Methods:** At the 28th gestational week plasma leptin was measured at fasting and following an oral glucose tolerance test (OGTT), basal insulin secretion rate (BSR;pmol/l) and total insulin secretion (TIS; pmol/l.3hrs) were evaluated by minimal modeling from frequently sampled OGTTs (AJP 270:E522, 1996). **Results:** Plasma leptin (ng/ml) was insignificantly higher in GDM (21.0±2.7) than in NGT (16.3±1.5) being increased in both groups vs. non-pregnant females (9.2±0.8; $p<0.05$). There were no differences between fasting and postprandial (OGTT) leptin concentrations in any group. BSR and TIS were increased ($p<0.05$) in GDM (BSR: 47.5±6.8, TIS: 36.4±4.1) compared to NGT (31.2±3.1, TIS: 26.5±3.0) reflecting marked insulin resistance in GDM. The overall pregnancy induced increase in body weight (kg) was slightly lower in GDM (11.2±1.9) than NGT (15.2±2.6) as was the birth weight (g) of their newborns (3624.1±247.2 vs. 3891.7±83.6). Plasma leptin correlated with BSR ($p<0.04$), TIS ($p<0.05$), basal glucose ($p<0.04$) and basal proinsulin ($p<0.05$) only in GDM. In addition leptin correlated in GDM with preconceptual BMI ($p<0.04$) and inversely with maternal weight gain during pregnancy ($p<0.04$) and birth weight of the newborns ($p<0.05$). 8 weeks after delivery glucose tolerance was normalized in GDM and plasma leptin decreased to 13.8±3.1 ($p<0.01$), while it showed a tendency to decrease in NGT (10.8±1.0). **Conclusions:** We found 1.) no difference between fasting and postprandial plasma leptin in pregnancy 2.) a positive correlation of leptin with insulin release and the preconceptual BMI, but a negative relationship with pregnancy related body weight gain and birth weight of the newborns in GDM and 3.) a decrease of plasma leptin in GDM after delivery and normalization of glucose tolerance. Therefore, plasma leptin is increased in insulin resistant GDM, being clearly associated with endogenous hyperinsulinemia, potentially hinting at a physiologic role for leptin during pregnancy in GDM.

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Serum leptin, plasma C-peptide and genetic mutations (IRS1 and β_3-adrenergic receptor) in gestational (GDM) and non-insulin-dependent (NIDDM) diabetic pregnant women.

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Aim: Leptin, the product of the *ob* gene, is thought to play a critical role in the regulation of adipose tissue mass, which may directly or indirectly influence reproductive function. Pregnancy is characterised by insulin resistance and hyperinsulinemia with advancing gestation and a close association between insulin and leptin has been reported. Since insulin resistance and hyperinsulinemia are even greater in GDM as well as in NIDDM women, we investigated serum leptin in diabetic and non diabetic pregnant women. **Materials and Methods:** We evalueted serum leptin and plasma C-peptide (CPR) levels by a RIA in 36 GDM, 14 NIDDM and 86 control pregnant women. Genetic mutations of the insulin receptor substrate-1 (IRS-1, codon 972) and β_3-adrenergic (codon 64) receptor were investigated in all groups by PCR and RFLP. **Results**: Serum leptin values (ng/ml) were lower in GDM (17.6±1.5, $p<0.05$) and NIDDM (16.1±3) than in control (23±2) women in late pregnancy. A significant correlation between serum leptin and CPR in late pregnancy was observed in both the control ($r=0.367$, $p<0.01$) and GDM ($r=0.4$, $p<0.01$) women. Grouping the CPR (pmol/L) values, according to the serum leptin (<10, >10-30, >30 ng/ml) values, we observed a significant increase in both control (CPR: 353±42, 667±88, 997±129) and GDM (CPR: 1002±184, 1222±175, 2360±395) women, being always the CPR values significantly greater in GDM than in control women. Moreover serum leptin values (ng/ml) were significantly higher in GDM women with gene mutation of IRS1 (34.5±2.9 versus 15.6±1.2, $p<0.001$) or IRS1 and/or β_3-adrenergic receptor mutation (24.6±4 versus 15.8±1.4, $p<0.01$). Finally serum leptin values were significantly correlated with pre-gestational BMI of the GDM women, while no correlation was found between leptin one hand and weight gain during pregnancy, birth-weight, age and therapeutic approach (diet versus insulin). **Conclusions:** These results suggest that genetic abnormalities may be involved in insulin resistance, hyperinsulinemia and increased serum leptin values in GDM and NIDDM diabetic pregnant women.

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PROINSULIN SECRETION IN NORMAL, OBESE AND GDM PREGNANCIES AFTER ORAL STIMULI.

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The role of beta cell dysfunction in pregnancy complicated by GDM is still controversial and the increase in insulin resistance induced by pregnancy is often taken as a major determinant of the syndrome. Moreover, associated factors such as obesity and age may prove confounding. **AIMS**: To evaluate insulin and proinsulin secretion during pregnancy, after oral stimuli, in normal weight, obese (BMI>25) and GDM women. **PATIENTS AND METHODS**: The diagnosis of GDM was made after a 100 g OGTT at an average of 200 days of pregnancy using Carpenter and Coustan criteria. Serum intact proinsulin was measured by a very sensitive and specific method after oral glucose and after a formula meal, in random order. **RESULTS**: Mean insulin levels were increased in obese women (n=7) versus lean (n=22) at time 0, 30, 60 and 120 min (17.4 ± 13 vs 8.3 ± 5; 113 ± 37 vs 72 ± 44; 134 ± 38 vs 90.9 ± 32 and 126 ± 84 vs 72.3 ± 35 µU/ml respectively) after oral glucose and at 30 min after the formula meal (181 ± 37.2 vs 88.5 ± 57 µU/ml) despite similar plasma glucose and proinsulin levels. By contrast, when comparing the β-cell responses in NGT (n=29) and GDM women (n=16), insulin levels were increased in the latter only at time 180 (104 ± 75 vs 56 ± 52 µU/ml) whereas proinsulin levels were increased at 60, 120 and 180 min both after oral glucose and formula meal (8.1 ± 5 vs 5.6 ± 4; 15.5 ± 10 vs 8.7 ± 8; 15.7 ± 11 vs 7.2 ± 8 pmol/l after OGGT and 9.7 ± 8 vs 4.5 ± 3; 18.4 ± 20 vs 7.8 ± 7; 11.7 ± 15 vs 4.7 ± 4.1 pmol/l after the formula meal). **CONCLUSIONS**: The increase in immunoreactive insulin observed in obese women during pregnancy is sustained by normal processing of insulin whereas beta cell function is impaired in GDM as ascertained by elevated proinsulin levels.

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ABNORMALITIES OF ISLET B-CELL FUNCTION AND INSULIN ACTION IN GESTATIONAL DIABETES MELLITUS: RELATIONSHIP TO BMI

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Aims: to determine, insulin sensitivity (S_I), glucose effectiveness (S_G), glucose tolerance (K_G) and insulin secretion in moderately obese and in lean GDM women (pre-pregnancy BMI≥27 and <27, respectively). Material and Methods: 16 GDM and 7 normal pregnant control (NPC) obese and 35 GDM and 20 NPC lean women were studied in late pregnancy. GDM and NPC were matched for BMI, age and WHR. An IVGTT (300 mg glucose/kg body weight, followed in 20 min by a 5 min infusion of insulin 6 mU/kg/min and blood sampled 14 times for 240 min was performed. S_I ($x10^{-4}min^{-1}$per µU/ml) and S_G (min^{-1}) were estimated by Bergman's minimal model. K_G ($min^{-1}x100$) was assessed between 8-19 min after glucose injection. First phase insulin secretion (µU/mlxmin) was expressed as the area under the insulin curve between 2-8 min. Disposition Index (DI) was calculated (S_I x first phase x 10^{-1}) to assess B-cell compensation for insulin resistance. Results: fasting glucose (mg/dl) and insulin (µU/ml) levels were higher in GDM than NPC, but only statistically significant for glucose. S_I was lower in GDM than NPC for each body habitus. S_G did not differ according to glucose tolerance status but tended to be higher in NPC than in GDM. First phase insulin was reduced 47% in lean GDM *vs* lean NPC and reduced 36% in obese GDM *vs* obese NPC. DI revealed even greater reduction in B-cell compensation for insulin resistance in GDM: 71% for lean GDM *vs* lean NPC and 71% for obese GDM *vs* obese NPC.

	Lean		Obese	
Variable (mean±SD)	**NPC**	**GDM**	**NPC**	**GDM**
ff. glucose	76±5	82±12[a]	80±9	89±14
ff. insulin	10±4	13±12	12±5	17±7
S_I	2.5±2.0	1.3±1.1[a]	1.8±2.2	0.6±0.5[b]
S_G	0.023±0.008	0.017±0.007	0.021±0.010	0.016±0.004
First Phase	781±608	411±269 [a]	831±485	532±269
"DI"	1.53±1.14	0.44±0.40[a]	1.07±0.88	0.31±0.40[a]
K_G	1.89±0.33	1.19±0.25[a]	3.3±0.82	2.05±0.36[b]

[a]*p<0.05, GDM vs NPC;*[b]*p<0.05, lean vs obese*

Conclusions: thus, we found that GDM are more insulin resistant than BMI-matched NPC, but obesity when present adds to the insulin resistance of GDM. Regardless of whether they are lean or obese, GDM have reduced B-cell compensation for insulin resistance. As the reduction in DI *vs* BMI-matched normal pregnant is exactly the same, we can conclude that the B-cell defect seems to be the major one in GDM whatever their BMI is.

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COST-EFFECTIVENESS OF SCREENING TEST AND METABOLIC MANAGEMENT OF GESTATIONAL DIABETES.

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Aims: this study was performed to evaluate the cost-effectiveness of a screening program for the diagnosis GDM; in women with GDM we also evaluated the cost-effectiveness of the intensive metabolic management for reduction of neonatal-maternal morbidity. **Methods:** we retrospectively compared 2 different groups of pregnant women, "screened" and "unscreened" for GDM, who delivered at the same hospital in two different periods. For the analysis of costs the tariffs of the Italian Public Health System were used; the costs obtained have been expressed in Euro. **Results:** women not screened for GDM included 4035 cases and the "screened" group included 1338 cases. Prevalence of GDM was 2.25% in the unscreened group and 6.68% in the screened group ($p<0.001$). GDM patients of the screened group showed significantly ($p>0.001$) lower incidence than the unscreened group for preterm delivery (29%vs 16,6%), Cesarean sections (48% vs 33%) and macrosomia (LGA) (55% vs 22%). The cost of screening per case of GDM in universal screening and in a group without lower risk women (selected screening) was Euro 424.4 and Euro 406, respectively. In a higher risk group, such as prepregnancy obese women, we have a cost per case of GDM of Euro 213 and the estimated cost, if GDM was diagnosed directly by OGTT (without screening test), was about Euro 141,5. The mean cost of intensive metabolic management per case of GDM of screened group, was Euro 319,4. **Conclusions:** universal screening is a useful instrument to identify women with gestational diabetes; "selected screening" in comparison with "universal screening" allows a 5% financial saving for each individual case of GDM diagnosed. In obese women the diagnosis for GDM made directly by OGTT unstead of using the preliminary screening test, allows a saving of c. 30%. Finally, once the diagnosis of GDM is made, metabolic management saves money in relation to the reduction of Cesarean section (Euro 1,728 for every Cesarean section avoided), preterm deliveries and neonatal morbidity (Euro 3,088 for each newborn with significant affections)

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GLUCOSE TOLERANCE AFTER PREGNANCY WITH GDM IN 72 WOMEN.
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AIM: To evaluate glucose tolerance 5 years after pregnancy complicated by GDM. **MATERIALS AND METHODS**: 172 pregnancies complicated by GDM were followed at the Diabetes and Pregnancy Outpatient Clinic of the San Raffaele Hospital in 1988-1993. Carpenter and Coustan criteria were used for diagnosis of GDM and WHO criteria for the OGTT at follow-up. **RESULTS**: Two women required insulin since after delivery. At present 93 women were contacted by phone and none had known diabetes, 70 women underwent an OGTT at the follow-up visit (75% of the contacted patients) and 11 refused to participate in the study. The mean age at the time of the follow-up visit was 37 ± 4.8 years (range 28-47) and time from the index pregnancy was 5.5 years (range 3-8.8). Mean prepregnancy BMI was 23.5 ± 4.6 and mean BMI at follow-up was 24.2 ± 4.2. Child birth weight was 3300 ± 509 g (range 1740-4340) at a mean gestational age of 39 ± 2 weeks. Of the 70 women who completed the follow-up visit 20 (28.6%) had abnormal glucose tolerance and 4 out of 20 had diabetes. No differences were observed between women with or without abnormal glucose tolerance for the following variables: prepregnancy BMI, BMI at follow-up, weight gain during pregnancy, parity, weight of the newborn, gestational age at delivery, cholesterol, triglycerides, HbA1c levels, birth weight of the mother, maximum weight during life, family history of diabetes, hypertension and cardiovascular disease. Women who developed abnormal glucose tolerance showed a trend for higher blood glucose levels at 120 and 180 min at the OGTT during pregnancy. **CONCLUSIONS**: Northern Italy has one of the lowest prevalence in Europe of both type 1 and 2 diabetes, nevertheless the diagnosis of GDM even in this population is associated with a high prevalence of abnormal glucose tolerance in the following years, 8.3% of diabetes and 22% of IGT compared to 4.4% and 3.7% respectively ascertained in a population study of a decade older women.

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PRIOR GESTATIONAL DIABETES: TYPING AND CHARACTERISTICS OF DIABETES MELLITUS AT FOLLOW UP AFTER 8 YEARS.
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Aims: To study prevalence and characteristic features of different forms of diabetes mellitus at follow-up in women with prior gestational diabetes (GDM). Patients and methods: A cohort (n=211; mean age: 38.5 ± 6.5 [± SD] yrs; BMI: 26.6 ± 5.5 kg/m^2; 140 first insulinized [GDM-I], 71 on diet [GDM-D] during gestation) has been traced 8.0±2.5 yrs after delivery. Beside filling in a questionnaire physical examination and laboratory investigations were performed. Results: Altogether 103 women (49%) could be reclassified (75 g oGTT or fasting / postprandial glucose using a test meal; [WHO criteria] or known diabetes, treated by oral hypoglycaemics or on insulin) with glucose intolerance (GI = diabetes mellitus + IGT; GDM-I vs GDM-D: P <0.01; OR: 4.47 [95% Confidence Interval: 1.73 – 11.55]); 88 persons had diabetes (43 on insulin; GDM-I vs GDM-D: P<0.001; OR: 12.78 [95% CI: 1.65 – 98.89]). Twenty two women with prior GDM were diagnosed as type 1 diabetes (GI on insulin at follow up + GADA or ICA positivity; 21% of GI cases), 66 women as type 2 diabetes: (22 on insulin, 24 on oral therapy, 20 on diet); 15 cases had IGT. To differentiate between diabetes types near-normal prepregnancy BMI (P<0.05), younger age (P<0.05), first pregnancy (P<0.05), earlier time of diagnosis (P<0.05) and earlier start of insulin therapy during gestation (P<0.0001) were predictors for type 1 diabetes using ANOVA. A high frequency of hypertension (23%; measured high blood pressure [≥160/95mmHg] and/or antihypertensive therapy) was a characteristic feature of the whole cohort (highest prevalence found in GI cases of the GDM-I group, not differring significantly from retyped IDDM cases [37 vs 33%; P:NS]). Similarly, no difference in the high prevalence (41 to 68% in different groups) of positive family history of diabetes could be proven. Conclusion: GDM and insulinization during pregnancy together are strong predictors of GI later in life. Heterogeneity and overlapping of some characteristic features / phenotypes found at retyping proves that GDM may be an early manifestation of a multimetabolic cardiovascular syndrome dominated later by diabetes mellitus (type 2) in most of the cases or complicating an autoimmune disease (type 1 diabetes or LADA).

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Clinical Issues

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Glucose control in UKPDS type 2 diabetic patients with and without autoantibodies to islet-cell cytoplasm and glutamic acid decarboxylase
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Aims: To assess the association of islet-cell antibodies (ICA) and glutamic acid decarboxylase antibodies (GADA), in type 2 diabetic patients, with glucose control. **Materials:** HbA_{1c} was measured annually in 4545 newly diagnosed type 2 diabetic patients with fasting plasma glucose > 6mmol/L of whom 80% were neither ICA nor GADA positive (+ve), 16% were either ICA or GADA +ve and 4% were both. **Methods:** Patients were randomly allocated to conventional policy primarily with diet, to sulphonylureas or to insulin (main randomisation (MR), n=3867). When fasting plasma glucose was above 15mmol/L (primary diet failures (PDF), n=678), patients were randomised to sulphonylureas or to insulin. The association of ICA/GADA status and allocated policy with median HbA_{1c} over 10 years from randomisation was examined in a two way analysis of variance. **Results:** Patients both ICA and GADA +ve had significantly *(p<0.0001)* higher median (IQR) HBA_{1c} *(MR:8.6(7.4,9.8);PDF:9.2(8,10.3))* than patients either ICA or GADA +ve *(MR:7.5(6.5,8.6);PDF:8.2(7.2,9.5))* and patients neither ICA nor GADA +ve *(MR:7.1(6.3,8.2);PDF:7.5(6.7,8.6))*; patients either ICA or GADA +ve had greater median HbA_{1c} than patients neither ICA nor GADA +ve. In the MR, patients allocated to diet had significantly *(p<0.0001)* higher median (IQR) HbA_{1c} *(7.8(6.8,8.7))* than patients allocated to sulphonylureas *(6.8(6.1,8))* or insulin *(7.1(6.3,8.1))*. **Conclusions:** Glucose levels of patients with ICA and/or GADA are less well-controlled than levels of patients without these antibodies. The change in glucose control induced by intensive policy with sulphonylurea or insulin was similar irrespective of ICA/GADA state.

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RELATIONSHIP OF CLINICAL MANAGEMENT WITH QUALITY OF LIFE IN ADOLESCENTS WITH IDDM.
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Aims: An international study involving 17 countries including Europe, Japan and North America was conducted to study the association between quality of life (QOL) and clinical management for children/adolescents with type 1 diabetes and their parents. HbA1c and clinical data were collected and QOL was assessed in young people by the Diabetes Quality of Life Questionnaire - DQOL (subscales: impact, worries, satisfaction, and health perception); and family burden by multidimensional questionnaires to parents and health professionals. Materials and Methods: 2,077 adolescents gave blood samples (boys 1,073, girls 1,004); median age 14.0 years (range 10-18 years); median diabetes duration 5 years (range 0-17 years); median HbA1c 8.5% (4.8-17.4); 1,984 adolescents completed questionnaires. Results: Adolescents with lower HbA1c had higher QOL (p=0.01); and lower parental (p=0.0001) and health professional assessment of burden (p=0.0001). QOL was significantly higher for younger than for older adolescents (p=0.0001), and for boys than for girls, in both age groups. Nonetheless, parental burden was greater in the younger, and in boys of both age groups. QOL was unrelated to duration of diabetes (p=0.33), although HbA1c rose with age. There was no significant relationship between number of insulin injections per day (p=0.61), or use of premixed insulin (p=0.31), with QOL for the adolescent, nor family burden as assessed by parent (p=0.61 and 0.95), or by health professional (p=0.58 and 0.61). Hypoglycaemia related to health professional burden (p=0.006), but not to QOL of adolescent or parental burden. Body mass index (BMI) was strongly and inversely related to adolescent rated QOL (p=0.005), and particularly to worries, satisfaction and health perception subscales. It was not associated with impact or with family burden (p=0.13 and 0.78). Conclusions: Good HbA1c levels are associated with better QOL for adolescents and lesser perceived burden by parents and professionals. QOL is unrelated to number of insulin injections, use of premixed insulin, or to hypoglycaemia (except for health professional burden). Differing concerns (hypoglycaemia associated with professional burden and BMI associated with adolescent QOL ratings) illustrate different priorities for different members of the adolescent/family/professional triad involved. Greater attention should be given to BMI as a source of QOL impairment.

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Nicotinamide and intravenous insulin administration in newly diagnosed type 1 diabetes mellitus

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Several attempts have been performed to preserve β-cell from destruction by applying experimental therapies in newly diagnosed DM1, including insulin and nicotinamide. **Aim**: to investigate the effect of i.v. insulin therapy combined with nicotinamide in the metabolic control and β-cell function of newly diagnosed DM1 subjects in comparison with intensive insulin therapy and nicotinamide alone. **Patients and methods**: 34 newly diagnosed DM1 patients were included. After the correction of initial metabolic disturbances, subjects were randomly assigned within 72 h after admission to three different groups : (i) Intensive insulin therapy plus placebo (C, n=12), (ii) Intensive insulin therapy plus nicotinamide, 700 mg t.i.d (NIC, n=11), and (iii) 72 h i.v. insulin followed by intensive insulin therapy plus nicotinamide, 700 mg t.i.d (NIV, n=11). Follow-up 12 months. GAD, IA-2 and IAA antibodies were measured. C-peptide was measured basally and after 2, 4, 6, 8 and 10 min. of 1 mg i.v. glucagon. HbA_{1c}, glucagon test and antibody measurements were determined initially and at months 1, 3, 6, 9 and 12. **Results:** HbA_{1c} remained within normal values along the study in all 3 groups during the follow-up (n.s). Neither fasting-c peptide concentration nor the AUC of stimulated c-peptide showed significant differences between groups of treatment during the study. After 1 month, max-stimulated c-peptide was significantly higher in the NIV group when compared to C group (0.57±0.21 v.s. 0.34±0.13 nmol/l, $p<0.05$). This value in NIC group was, on average, 51 % higher than in C group (n.s.). At diagnosis GAD and IA2 positivity was observed in 10/12, 3/12; 8/11, 4/11 and 10/11, 4/11 of C, NIC and NIV groups respectively (n.s.). Antibody titers display a similar behaviour in all groups during the follow-up,. **In summary**, intravenous insulin administration shortly after diagnosis plus nicotinamide and nicotinamide alone are as effective as intensive insulin therapy in preserving β-cell function in newly diagnosed DM1 during the 1st year of treatment.

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PANCREATIC EXOCRINE INSUFFICIENCY AND TYPE 2 DIABETES ARE STRONGLY ASSOCIATED

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Aims: An association between Pancreatic Exocrine Insufficiency (PEI) and both Type 1 and Type 2 diabetes has been suggested, but previous studies have been limited by their small size and the possibility of selection bias. The availability of faecal elastase 1 measurement as a specific test of pancreatic function has facilitated examination of this association in population-based studies. The aim of this study was to quantify the strength of the association between PEI and Type 2 diabetes. **Materials and Methods:** Population-based study using a random sample of 526 Type 2 diabetic patients (age: 63±8 yrs; 63% males) selected from a local diabetes register in Cambridgeshire, UK, and 526 non-diabetic controls, individually matched for age, sex, and practice. In controls, diabetes was excluded by medical record search and HbA_{1c} measurements. Samples of faces were collected and elastase 1 concentrations were measured centrally by ELISA (ScheboTech, Germany). **Results:** PEI (elastase <100 µg/g stool) was found in 11.8% of cases and 3.8% of controls, yielding a crude age-sex-adjusted odds ratio (OR;95%CI) for PEI of 3.4 (2.1-5.9). After adjusting for potential confounders (BMI, alcohol intake, history of gastrointestinal diseases) using logistic regression models, the OR for PEI was 3.8 (2.3-6.8). Among patients with diabetes, PEI was associated with HbA_{1c} (OR: 1.32; 1.04-1.68). However, no significant association was found with BMI, diabetes duration, or presence of known complications, in particular, peripheral neuropathy assessed by vibration sensitivity. **Conclusions:** Type 2 diabetes is associated with a threefold increased risk of PEI. Further studies are needed to assess the pathogenesis and clinical implication of this finding.

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COULD TUMOR MARKER CA-19-9 BE OF USE IN PRACTICING DIABETES MELLITUS (DM)?

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The purpose of this study is to find out if the tumor marker CA-19-9 could be used as an indicator for the diagnosis of cancer of the pancreas before it is inoperable.

Methods: We measured CA-19-9 in all patients, who were diagnosed to have hyperglycaemia for the first time with symptoms of type 2 DM. Computerized tomography of the pancreas was done to the patients with CA-19-6 > 80u/ml (normal value<37u/ml). Those with CA-19-9 < 80u/ml were reexamined after 2 months. We examined 122 patients (64 men aged 60+5 and 58 women aged 58+8). The patients were asked for family history of DM and examined for retinopathy. For those with positive computerized tomography MRI was ordered.

98 patients with type 2 DM diagnosed at least 5 years ago and well matched for age and sex were used as a control group.

Results: These data are preliminary and we carry the study on. 3 men had CA-19-9 > 5000u/ml and were diagnosed to have pancreatic cancer. These patients had epigastric pain, but they visited us for DM. 1 man and 1 woman had CA-19-9 980u/ml and 1000u/ml respectively and no other complaint except DM, they were proved to have cancer of the pancreas inoperable. 2 men and 3 women had high CA-19-9 but less than 100u/ml; in all of them the computerized tomography was normal. None of the patients with cancer had family history of DM, or retinopathy. In the control group 2 men and 3 women had high CA-19-9 but less than 100u/ml and no abnormal findings in the computerized tomography of the pancreas. These data are not amenable for statistical analysis, but can give some indication for measuring CA-19-9.

Conclusion: Patients with recent hyperglycemia, without family history of DM and without retinopathy should be checked for cancer of the pancreas hopefully operable, by ordering CA-19-9.

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THE SIGNIFICANT ASSOCIATION OF ELEVATED FASTING GLUCOSE VALUES WITH CARDIOVASCULAR DISEASES

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The diagnostic criteria for diabetes mellitus have been re-examined 1998, and, especially with respect to the increased cardiovascular risk associated with type II diabetes, diagnostic fasting blood glucose values were lowered to 6,1 mmol/L. Impaired fasting glucose tolerance was defined by glucose values between 5,6 to 6,1 mmol/L.

The aim of our evaluation was to investigate the correlation of the various fasting blood glucose levels with cardiovascular diseases.

Methods: For this purpose we have performed a correlation analysis of all fasting glucose measurements performed between 1995 and 1999 (13.474 measurements) with clinical diagnosis according to the VESCA code (3515 cardiovascular diagnosis, 9959 non-vascular disorders). Mean fasting glucose values were significantly higher in the group with cardiovascular diseases (angina, myocardial infarction, stroke, peripheral vascular disease) (6,2 ± 2,2 mmol/L) than in the group with non vascular disorders (5,8 ± 2,3 mmol/L) (p=0,001). Regarding the various degrees of fasting glucose levels a significant difference between cardiovascular and non-vascular diseases was found for fasting glucose levels higher than 6,1 mmol/L (p=0.001), and thus for manifest diabetes, as well as for impaired fasting glucose tolerance, where glucose levels were significantly higher in cardiovascular diseases (p=0,001). The difference was absent for fasting glucose values below 5,5 mmol/L.

Results: The results of our study thus confirm the clinical importance of the new diagnostic criteria for type II diabetes with respect to cardiovascular events.

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DO CIRCULATING CYTOKINES RELATE TO BONE METABOLISM IN DIABETIC POSTMENOPAUSAL WOMEN ?

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Cytokines are known to participate in the pathogenesis of insulin resistance as well as of postmenopausal osteoporosis.Although these are mostly paracrine effects,we wished to explore the potential contribution of circulating IL-6 and TNF-α levels,the main endocrine cytokines.We studied 24 postmenopausal women with type 2 diabetes (DM) and 16 without diabetes (HL). Their age (mean ±1 SD) was 57.2±6.2 and 56.7±7.4 yrs, the interval since menopause (YSM) 10.2±5.4 and 7.8±4.9 yrs and the BMI 26.6±4.1 and 27.2±4.6 Kg/m^2 respectively. Duration of diabetes was 9.5±4.8 yrs. Bone mineral density (BMD) in g/cm^2 was measured at L2-L4 vertebrae and proximal femur by Dual Energy X-ray Absorptiometry and serum IL-6, TNF-α levels by ELISA.Femoral neck BMD of DM (0.76±0.08) was lower than of HL (0.87±0.15), p=0.01,while that of L2-L4 did not differ significantly.TNF-α levels (ng/ml) were significantly higher in DM (4.6±1.1) than in HL (3.27±1.1), p<0.001, whereas IL-6 levels (ng/ml) did not differ (1.47±1.2 vs.1.45±1.04). A positive correlation was observed between IL-6 and BMI of DM (r=0.74,p<0.001) as well as between TNF-α and menopausal age (r= 0.4, p<0.05). IL6 and TNF-α levels of DM were negatively correlated to serum osteocalcin levels (p<0.05 and <0.01 respectively).**In conclusion,** BMD values of mixed bone are lower in diabetic than in normal postmenopausal women, while those of trabecular bone do not differ. Circulating IL-6 and TNF-α levels do not directly relate to BMD behavior in postmenopausal women. TNF-α levels, however were elevated in diabetic postmenopausal women, which might contribute to the development of insulin resistance.

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LIVER FAT CONTENT, INSULIN ABSORPTION AND ACTION AS DETERMINANTS OF INSULIN REQUIREMENTS IN TYPE 2 DIABETES

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Aim: To determine the causes of interindividual variation in insulin requirements. **Methods**: We recruited 20 type 2 diabetic patients, who had had stable glucose control (HbA_{1c} < 8.0 %) for over 1 year on combination therapy with bedtime NPH and metformin. Liver fat content (proton spectroscopy), visceral fat (MRI) insulin sensitivity (6 hr 0.3 mU/kg·min euglycemic insulin clamp combined with 3-[^{3}H]-glucose), insulin absorption (increase in free insulin over 8 hrs after a fixed dose of regular insulin), action of injected insulin (glucose infusion rate required to maintain euglycemia), weight and body composition were determined. **Results**: *Variation in parameters*: insulin dose range 10-176 IU (mean 42 IU, fold variation 17.6x); or 0.13-1.39 IU/kg (0.44 IU/kg, 10.7x); weight 67-127 kg (91 kg, 1.9x), liver fat 2-28 % (12 %, 14x), visceral fat 179-2053 cm^3 (1114 cm^3, 11.5x), absorbed insulin /8 hrs·ffm 68-720 (372, 10.6x), suppression of glucose production -18 to -142 % (-67 %, 7.9x), suppression of FFA by s.c. insulin 59350-452300 µmol/lxmin (7.6 x), and by i.v. insulin 38190-385600 µmol/lx min (10.1 x). Both the *action of absorbed insulin*, determined from suppression of FFA/ 8hrs (r=0.71, p<0.001) and the glucose infusion rate (r=-0.56, p<0.02) were significant determinants of the *daily insulin dose (IU/kg)*. Visceral fat volume (r=-0.77, p<0.001) and simply BMI (r=-0.72, p<0.005) were significantly related to the amount of insulin absorbed. The best determinant of hepatic sensitivity to insulin was the % hepatic fat (r=0.71, p<0.001), which in turn was significantly related to total fat mass (r=0.73, p<0.001) but not the ratio of visceral to subcutaneous fat. The ability of i.v. insulin to suppress FFA was closely related to the ability of s.c. insulin to suppress FFA (r=0.88, p<0.001) implying that 77 % of the variation in insulin action on antilipolysis was due to variation in insulin sensitivity and 23 % to the combined effects of variation in insulin absorption, day to day variation in FFA concentrations and methodology. **Conclusions**: The major reason for interindividual variation in insulin requirements in type 2 diabetes is that in insulin sensitivity rather than absorption. The data also suggest that hepatic fat content influences hepatic insulin sensitivity.

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DO AFRICAN AMERICAN (BLACK) CHILDREN AND ADOLESCENTS TREATED WITH INSULIN HAVE TYPE 1 OR TYPE 2 DIABETES?

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We have previously reported that only 75% of Black children and adolescents diagnosed clinically with insulin-dependent diabetes have autoantibodies to the β cell (islet cell antibodies measured in human pancreas, autoantibodies to GAD and IA2) at onset compared to 95% of Whites. It is unclear whether Blacks without these traditional autoantibodies present different demographic or clinical characteristics at onset. In order to clarify this issue, we reviewed medical charts in all Black children and adolescents (n=40) who were less than 19 years of age, diagnosed as having IDDM between 1983-1985, 1989 and 1996-1997 at Children's Hospital of Pittsburgh. The information obtained was compared between Blacks with at least one of the traditional autoantibodies (type 1 or group A) and those without any (type 2 or group B).

	Group A (n=27)	Group B (n=13)	p-value
Mean age at onset	8.8	12.9	0.006
Sex (M/F) (%)	41/59	38/62	0.89
Obesity (BMI ≥ 90%ile)	29.6	61.5	0.05
Presence of ketones (%)	81.8	66.7	0.40
DKA (%)	62.5	33.3	0.09
Family history of diabetes (%)	66.7	61.5	1.0
Parent with diabetes (%)	8.3	30.7	0.09

Blacks without traditional autoantibodies had an older age at onset and a higher prevalence of obesity. They had a lower prevalence of DKA at onset and a higher prevalence of having a parent with diabetes but perhaps due to the small sample sizes, these differences were not statistically significant. It is important to note that 38% of Blacks without traditional autoantibodies (Group B) had ICA directed to a rat islet cell antigen (all of them with ketones). In contrast, 50% of the Blacks without any antibodies also presented ketones, a hallmark of IDDM. On the other hand, 30% of Blacks with traditional autoantibodies were obese. Thus, presence of ketones or DKA does not predict the presence of traditional autoantibodies and obesity does not exclude type 1 diabetes in Black children and adolescents.

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THE ROLE OF VIRAL INFECTIONS IN TYPE 1 DIABETES IN SOUTH AFRICAN BLACKS

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Aim: The study aimed to evaluate the role of selected viruses in the pathogenesis of type 1 diabetes in a group of South African Black subjects.

Materials and Methods: The study group comprised 54 type 1 diabetic subjects and 38 healthy blood donors. In each group, the prevalence of antibodies to glutamic acid decarboxylase (GAD), enteroviruses (IgM) and hepatitis C virus was determined by ELISA. Presence of viral RNA in DNAse-treated RNA extracted from plasma was determined by reverse transcriptase polymerase chain reaction (rtPCR) for hepatitis G, human endogenous retrovirus IDDMK$_{1,2}$22 (IDDMK$_{1,2}$22) and enteroviruses. The sequence of selected IDDMK$_{1,2}$22 products was determined in a cycle-sequencing reaction.

Results: 31.25% of the diabetic subjects had anti-GAD antibodies whereas these were not detected in the controls (p<0.05). None of the study subjects tested positive for enteroviral IgM antibodies or Hepatitis C antibodies. 25% of the diabetic subjects were positive for Hepatitis G compared to 38.2% of blood donors (p=0.72). 68% of type 1 diabetics were positive for IDDMK$_{1,2}$22 compared to 65.6% of blood donors (p=0.83). The DNA sequence of the IDDMK$_{1,2}$22 fragments amplified from the plasma of 2 diabetic subjects corresponded with GenBank sequence AFO12335 whereas that of one blood donor differed in at least 3 bases, identifiable as the loss of an *Sph* 1 restriction site. Subsequent restriction digest testing of all positive IDDMK$_{1,2}$22 samples revealed a dimorphic *Sph* 1 fragment mixture in all cases.

Conclusions: No evidence for infection with enteroviruses, hepatitis C or hepatitis G was found in association with type 1 diabetes in this group of South African Blacks. IDDMK$_{1,2}$22 expression appears to be widespread with no specific association with type 1 diabetes. The rtPCR used to demonstrate IDDMK$_{1,2}$22 mRNA, additionally amplifies a related retroviral fragment in all cases.

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CHILDHOOD IDDM INCIDENCE IN EASTERN BULGARIA - A 25-YEAR SURVEILLANCE (1973-1997)

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Long continuous registration of newly-diagnosed IDDM children on the Balkans are relatively rare. **Aim:** The study aims to assess the figures and trends in the childhood IDDM incidence in the last 25 years in Eastern Bulgaria. **Materials and methods:** The childhood (0-14 years) IDDM incidence in Eastern Bulgaria (1/3 of the country's territory and population) was studied retrospectively for the years 1973-1981. Since 1982 an IDDM registry is working prospectively. The IDDM incident cases (standard criteria) are entered annually using as a first source of data the hospital files from the mandatory admission at diagnosis. The secondary source are the lists of endocrinologists responsible for the post-hospital management. In the last 9 years the registry participates in EURODIAB ACE and DIAMOND. Due to the centralised insulin supplementation the case ascertainment is as high as 97.4% for the retrospective and 98.2% for the prospective part of the study. **Results:** The registry identified overall 931 newly-diagnosed IDDM children. The mean annual incidence was 6.44/100 000 (95%CI 6.02÷6.86) and for the last 10 years - 7.78/100 000 (95%CI 7.10÷8.66). The girls were affected more often than boys - 6.48 vs. 6.39/100 000 ($p>0.05$). The IDDM incidence was significantly higher among urban children - 7.39 vs. 4.86/100 000 ($p<0.001$), similarly when analysing by age-groups. Older children (10-14 y.) were at greater risk of developing diabetes than younger ones (0-4 y.) - 8.74 vs. 3.66/100 000, resp. ($p<0.001$). The mean annual incidence was highest in Varna county, the most densely populated - 7.33, and lowest in the least populated Bourgas county - 5.05/100 000. Poisson regression analysis revealed linear trend of increase in the incidence dependent on area of residence ($p<0.001$) and calendar year at diagnosis ($p<0.01$). Overall 69 (7.41%) children had Ist degree relatives with IDDM with no significant difference by area of residence. **Conclusions:** There is a clear trend of increase in the IDDM incidence in Eastern Bulgaria confined to the urban children which points to exogenous factors in diabetes etiology.

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THE GIESSEN-BAD OEYNHAUSEN FAMILY STUDY: IMPROVED PREDICTION OF TYPE I DIABETES IN RELATIVES BY COMBINING ISLET AUTOANTIBODIES IN A DUAL STEP MODEL

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To determine the value of combined antibody screening for prediction of type I diabetes in a low incidence cohort we prospectively studied 882 first-degree relatives (485 parents, 382 siblings, 15 offsprings) for up to 11 years unselected for islet cell antibodies (ICA). During the study period 16 individuals developed diabetes. The first serum sample obtained at study entry was analyzed for ICA, antibodies to insulin (IAA), GADA and anti-IA-2ic. A Cox proportional hazard model considering the joint effects of all baseline variables selected the four antibodies and the specific family history as significant risk confounding factors ($p<0.05$). Further analysis by Kaplan-Meier lifetables confirmed a significantly increasing risk of diabetes with the number of autoantibodies present ($p<0.001$) and in accordance with the Cox model relatives with more than one affected family member and siblings/offsprings vs. parents were at increased risk of IDDM ($p<0.05$). Beside technical problems a screening strategy based on initial ICA testing has the potential to miss ICA negative subjects among future cases of type I diabetes and we therefore set out to evaluate an alternative approach using a dual step strategy with a combination of GADA and anti-IA-2ic for initial screening followed by retesting of positive individuals for ICA and IAA. The combination of GADA and anti-IA-2ic for primary screening (step 1) proved to be more sensitive identifying 94% of future cases of type I diabetes compared to 81% using ICA as initial test and this antibody combination identified 93% of those individuals with ICA of 20 JDF or more. Retesting of positive individuals for ICA and IAA (step 2) significantly improved the positive predictive value confering a risk of diabetes for siblings and offsprings with more than 2 antibodies within 5 years of 67% (95%CI: 39-90). We conclude that the prognosis of contracting IDDM in relatives is strongly related to the number of autoantibodies present but the family history should be additionally considered for individual risk assessment. The proposed screening strategy could overcome the problems of the ICA and IAA assays for large-scale screening and in the present study it allows 5-years risk estimates up to 67% identifying 94% of future cases of type I diabetes.

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KARLSBURG TYPE I DIABETES RISK STUDY IN SCHOOLCHILDREN: FREQUENCY OF THYROID AND COELIAC AUTOANTIBODIES IN CHILDREN WITH DIABETES-ASSOCIATED AUTOANTIBODIES

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Aims: In this study we examined the occurrence of thyreoglobulin- (TgAb), thyreoperoxydase- (TPOAb), gliadin-IgA- (AGA) and endomysial-IgA-antibodies (AEA) in children of a normal population, positive for autoantibodies (AAbs) against glutamate decarboxylase (GADA), protein tyrosinphosphatase (IA2A), insulin (IAA) and/or islet cell cytoplasmic antigens (ICA). **Materials and Methods:** 9,419 children, aged 6-17 years, were tested in primary screening for GADA, IA2A and IAA by 125I-antigen binding and for ICA immunohistochemically. In children, positive for at least one AAb by re-examination, TgAb and TPOAb were determined using radioimmunoassays and AGA and AEA by indirect immunofluorescence. **Results:** 209 of the re-examined children had at least one AAb. 45.3% of them were positive ($\geq 99^{th}$ centile) for GADA, 28.2% for IA2A and 26.8% for IAA, and 29.2% for ICA (≥20 JDFU). 82.3% were positive for only 1 and 17.7% for two or more AAbs. 8.6% had TgAb and/or TPOAb, 7.2% AGA and 0.5% AEA. TgAb and/or TPOAb were more frequent in girls (11.5%) than in boys (5.2%) and in children with multiple (13.5%) vs. those with single diabetes-associated AAbs (7.6%). No child with multiple AAbs including IA2A was positive for TgAb or TPOAb. The frequency of TgAb and/or TPOAb was enhanced in children with GADA (10.5%), IAA (12.5%) and ICA (9.8%) vs. those with IA2A (1.7%; $p<0.05$). In contrast, the frequency of AGA was similar between boys (7.2%) and girls (7.5%) and slightly increased in those children with multiple diabetes-associated AAbs (10.8%) vs. single AAb positivity (6.4%). There were also no significant differences in frequency of AGA between children positive for GADA (9.5%), IAA (7.2%), IA2A (7.8%) or ICA (6.6%). One boy had increased AGA and AEA. He was also positive for GADA, IA2A and ICA, and developed diabetes 24 months after first AAb testing. None of the children had any symptoms of thyroid or coeliac disease. In controls the frequency of TGAb and/or TPOAb was 14.3% and for AGA 0.3%. **Conclusions:** The results confirm that IA2A have the highest Type I diabetes specificity among the diabetes-associated AAbs. The increased frequency of gliadin-IgA-antibodies but not of thyroid-AAbs in children with diabetes-associated AAbs compared to controls, suggesting a higher risk for future coeliac disease.

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KARLSBURG TYPE I DIABETES RISK STUDY IN SCHOOLCHILDREN - NO FURTHER DIFFERENTIATION OF RISK BY HLA-DQB1 GENOTYPING OF CHILDREN WITH MULTIPLE AUTOANTIBODIES

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Aims: Islet cell autoantibodies precedes onset of Type I diabetes in genetically predisposed patients. Thus this study was aimed to further differentiate the risk to develop diabetes by HLA-DQB1 genotyping of children primarily identified by combined autoantibody (AAb) screening. **Materials and Methods:** By re-examination of 203 AAb positive subjects screened in 6,155 schoolchildren, AAbs against glutamate decarboxylase (GADA), protein tyrosinphosphatase (IA2A), insulin (IAA) and islet cell cytoplasmic AAb (ICA) were determined above 99^{th} centile and ≥20 JDFU and HLA-DQB1 alleles were analyzed by DNA typing. **Results:** 2.71% (167/6,155) of probands were positive for one AAb specificity only. Among them subjects positive for GADA only ($\geq 99.9^{th}$ centile) or IA2A only ($\geq 99.5^{th}$ centile) have most frequently at least one diabetes-associated allele *0302 and/or *0201 ($p<0.05$ vs 135 AAb negative age-matched controls) whereas the dominant protective allele *0602 was absent. In contrast frequencies of associated/protective alleles of children positive for IAA only or ICA only did not differ from those of controls. 0.58% (36/6,155) were positive for ≥ 2 AAb specificities and defined as probands at increased risk. Among them 50% (18/36) were positive for two AAb specificities, 33.3% (12/36) for three AAbs and 16.7% (6/36) for all 4 AAbs. 83.3% (30/36) of these schoolchildren carry at least one associated allele but non of them has the protective *0602 allel. During the short follow-up of 44.5±9.5 months six probands (3m/3f) positive for three or four AAbs at high levels progressed to diabetes. Five of these six cases have at least one associated HLA-DQB1 allele. Thus the highest positive predictive values of 25% were calculated for children positive for GADA+IA2A+ICA or positive for all four AAb specificities. **Conclusion:** Multiple AAb positivity identifies subjects at risk for development diabetes in the general population and is strongly associated with the occurrence of the Type I diabetes-associated risk alleles. Thus risk for diabetes detected and differentiated by combined AAb analysis can not be further differentiated by HLA-DQB1 genotyping.

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INCIDENCE AND PREVALENCE OF TYPE I DIABETES MELITUS AMONG ADULT POPULATION IN LITHUANIA 1991-1997

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The aims of the study were to determine the incidence of insulin dependent diabetes mellitus (IDDM, type 1) in a prospective study of 15-39-year aged young adults and prevalence of type 1 diabetes mellitus among ≥15-year-aged Lithuanian population during the years 1991-1997. Two sources of information were used: urgent reports of onset and annual reports from all physicians responsible for diabetes care of all out-patient clinics of Lithuania. The incidence ascertainment was assessed by the capture-recapture method. During the 7-year period 769 new cases (496 males and 273 females) were identified. The average 7-year incidence rare was 9.992 per 100,000 (95% Poisson confidence interval was 9.150-10.911) for males and 5.601 (CI 4.974-6.306) for females, respectively (Chi square sex difference 41.013, df=4, p<0,001. Male/female ratio was 1.817. The highest age-specific risk of type 1 diabetes was observed around 25-34 years of age. Results of the regression models showed that the incidence of type 1 diabetes mellitus had tendency to increase among males and remain stable among females. The largest proportion of type 1 diabetes mellitus was diagnosed during full-winter (31.3%) and full-autumn (28.3%) and the lowest in summer (13.8%). No significant geographical and national variations were observed. On 31.12.1991 there were 2426 adult (≥15-year) patients with type 1 diabetes mellitus or 82.71 per 100,000 inhabitants (CI 79.46-86.08) in follow-up in Lithuania. On 31.12.1997 the number of patients rises to 3259 or 111.12 per 100,000, p<0,05. Age-adjusted prevalence rates in 1991 were 60.76 for males and 75.27 for females, and in 1997 - 127.74 and 95.82, respectively. In 1997 the domination of males was especially prominent in 35-39-year-aged group (229.13) and the domination of females - in 25-29-year-aged group (157.94). Our data shows the male predominance of type 1 diabetes mellitus in adults in Lithuania.

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THE RECENT TREND IN THE INCIDENCE OF TYPE 1 DIABETES IN UPPER SILESIA CHILDREN, 1989-1997 (POLAND).

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Aim: The purpose of the study was to provide current and reliable information about trends in incidence rates of Type 1 Diabetes in the Upper Silesia region in children aged 0-15 years in the period of 9 years. **Material and methods:** 528 cases with diabetes were ascertained independently during the registration period of 9 years. The study was made within the EURODIAB programme, according to the criteria and method accepted in it. **Results:** Incidence ratio in the examined population increased from 4,71 in 1989 up to 10,16/100 000/ year in 1997. In the analyzed Group no significant statistical differences dependent on sex were found. During the examined period the incidence ratio has significantly grown. It was observed that the extent of change varied in different age Groups. The youngest children (0-4 years old) showed the greatest increase of the incidence ratio from $1,09/10^5$ in 1989 up to $6,75/10^5$ in 1997. The highest incidence ratio was in the 10-14 years old age Group.**Conclusions:** In the examined period dramatic increase (more than 200 %) of the incidence ratio in the children's population (0-14 years old) in Upper Silesia was reported. The fastest increase of this ratio was established in the youngest children age Group (0-4 years old).

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Epidemiology of Type 1 Diabetes II

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Very low incidence of Type 1 diabetes in children in Romania

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According to the annual statistic data, the total population of Romania was 22.680.951 in 1997. The overall ascertainment-corrected Type 1 diabetes incidence in the age group 0 – 14 yr. was obtained from Bucharest (2.33 million inhabitants) and other 10 administrative counties, comprising a number of 5.36 million inhabitants. Alltogether, these 7.69 million inhabitants included in this study represent 33.9% of Romania's population. From this population, the number of children in the age group 0-14 yr. was of 1.58 million, representing 34.1% from the children in this age group. The age-corrected incidence of Type 1 diabetes was determined according with the EURODIAB ACE protocol for each county and for each year between 1988 and 1997. In this period we recorded 595 cases with the following main characteristics: 1. An overall slow increase of the number of cases, but with an important year to year fluctuation; the 328 cases recorded in the last 5 yr. (1993-1997) vs. only 267 cases in the first 5 yr. (1988-1992) represent a 22% increase; 2. The average incidence rate for the whole studied population in these 10 yr. was of 3.2/100000/yr. with the highest figure in Bucharest – 5.27/10000/yr. and the lowest in Mehedinti county – 1.55/100000/yr. This indicates a 4.5 fold geographic (between counties) incidence variation between the highest and lowest rate with any apparent explanation from ethnic distribution of population, alimentary habits or longitude/latitude of the counties; 3. It was a small but constant masculine predominance (3.47/100000/yr. vs. 3.25/100000/yr. in females); 4. Two thirds of the cases were recorded during the 6 coldest months of the year and only 1/3 in the warmer 6 months. This seasonallity is partially explained by the high incidence of viral and bacterial infections during the cold seasons which were recorded in 92% of the cases with onset in the cold seasons and just in 35% of the cases recorded in the warm seasons. **Conclusions:** With an overall 10 yr. incidence of 3.2/100000/yr. Romania is an European country with a very low risk for Type 1 diabetes in the age group 0 – 14 yr. which is explained not only by the genetic background but perhaps by some non-identified environmental factors

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SEX DIFFERENCES IN THE INCIDENCE OF TYPE 1 DIABETES IN TWO ITALIAN REGIONS: LAZIO AND SARDINIA

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Differences in the incidence of Type 1 diabetes have been often described, mainly in Northern Europe where a slight increase in the male/female ratio has been reported. **Aims:** In the present study we investigated a number of variables among recent onset Type 1 diabetics aged 0-14 from two Italian registers, Lazio and Sardinia, which have shown low and high incidences over the past eight years, respectively. These two regions share similar environmental influence and take part in the Eurodiab subarea A study since its implementation in 1989. In Lazio the mean yearly incidence rate per 100,000 (< 15 years of age) is 8.36 and in Sardinia 44.78. **Materials and Methods:** Patients diagnosed between 1989-1996 were subdivided in 3 age groups (0-4, 5-9, 10-14) and differences per age group and per year have been evaluated over the period 1989-1996. **Results:** In the Lazio region male incidence figures per 100,000 were 7.00 for the 0-4 age group, 10.66 for the 5-9 age group and 7.51 for the 10-14 age group. No differences for sex prevalence in all age groups over the period of registration was found. In Sardinia, male incidence figures per 10^5 were 31.53 for the 0-4 age group, 47.40 for the 5-9 age group and 52.15 for the 10-14 age group. Overall there was a male prevalence in the age group 0-14 compared to females ($p<0.001$) which was mainly due to the very high male preponderance in the 10-14 group. **Conclusion:** In a region with high incidence of Type 1 diabetes, such as Sardinia, male prevalence is higher, while in a very close region such as Lazio where the incidence is much lower, this is not observed. Therefore, genetic factors, rather than environmental ones, play a major role in a given population in determining the high frequency of Type 1 diabetes.

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THE SEASONAL DISTRIBUTION OF BIRTH OF PATIENTS WITH INSULIN-DEPENDENT DIABETES MELLITUS IN SARDINIA

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Aims: Since a seasonality of birth of patients with Insulin-Dependent Diabetes Mellitus (IDDM), indicating environmental influences during fetal life on the risk for the disease, has been described in several European areas, we investigated whether such a phenomenon is present in Sardinia, an area with the highest IDDM incidence in the world together with Finland. **Materials and Methods**: We compared the monthly distribution of birth in the Sardinian general population with that of 425 IDDM patients (from the Sardinian IDDM Registry) under 15 years of age, diagnosed during 1989-1992, born between the years 1974 and 1992. Statistical significance of the deviation of the observed to the expected numbers of cases was calculated using the test for seasonality of events designed by Walter and Elwood. **Results**: The two distributions did not differ from each other, thus excluding a seasonal pattern. **Conclusions**: At variance with UK-Leicester, Iceland, Sweden and The Netherlands, where a monthly pattern of births has been described, in Sardinia no clustering of time of birth for children with IDDM has been found. While seasonality at diagnosis may be related with environmental factors precipitating clinical onset of diabetes in subjects at a pre-clinical stage of the disease, seasonality of birth could reveal exposition to factors involved in initiation of the autoimmune process against β-cells early in life. From the present data we can conclude that, in Sardinia, no evidence exists of environmental factors initiating IDDM during pre- or perinatal life.

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THE INCIDENCE OF INSULIN-DEPENDENT DIABETES MELLITUS IN THE AGE GROUP 0-9 YEARS IN SIBERIA IS INCREASING

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Aims:To assess the connection between the age-at-diagnosis of insulin-dependent diabetes mellitus (IDDM) among siberian children diagnosed under 15 years of age and increasing incidence of IDDM at Siberia.
Materials and Methods: The epidemiological survey at Novosibirsk area have been conducted according to standards of WHO program "Diamond". The study period covered the years 1980-1997.
Results: The mean IDDM incidence was higher in 1992-1997 study period: 7,2±0,2 cases per 100 000 children (95% confidence interval (CI):6,8-7,6) than in 1986-1991 and 1980-1985 study periods where it was 4,8±0,2 (CI:4,5-5,1) and 4,1±0,4 (CI:3,3-5,0) accordingly. For the first time we have revealed the significant increasing of IDDM incidence both among children from big City and among children from smaller places. We have not found significant difference in IDDM incidence among 10-14 years old children at these study periods. But we have found significant increasing of IDDM incidence among children of 5-9 and 0-4 age groups. For the 5-9 years children the incidence has increased from 4,2±0,5 (CI 3,2-5,1) at 1980-1985 study period to 7,2±0,6 (CI 6,0- 8,4) at 1992-1997 study period. The mean IDDM incidence among 0-4 years children has increased from 1,8±0,2 (CI 1,4-2,2) in 1980-1985 study period to 5,9 ± 0,9 (CI 4,1-7,7) in 1992-1997 study period.
Conclusions: We suppose that the increasing of IDDM incidence in Siberia is caused by the negative influence of still unknown agents to children at the age from 0 to 9.

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SEASONALITY OF BIRTHS OF SLOVAK IDDM CHILDREN

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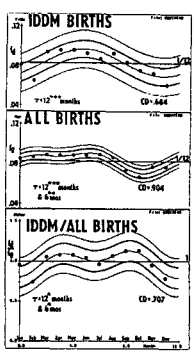
Aims: Seasonality of birth of IDDM children, with fewer births in winter, was found in Britain, not across other Europe including Slovakia. Analogical study is now presented from Slovakia on extended time interval. **Materials and Methods**: From Slovak National Register, 1186 children with IDDM born 1974-97, diagnosed 1989-97 were analysed *vs.* 1987676 children of general population. For each month, the month-length-standardized fraction of the yearly total equal 1 was calculated for IDDM (fd) and all births (fg). According to null hypothesis, either fraction has to be always 1/12, and fd/fg should be 1. Data were processed by cosinor regression. **Results**: For fd, significant (α=0.05) annual and for fg and fd/fg annual and semiannual cyclings were found (Figure). **Conclusions**: The birth rate of Slovak IDDM children was, compared to population expectations, significantly decreased in December-February and significantly increased in September.



Support: Eurodiab TIGER.

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NO INCREASE OF TYPE 1 DIABETES MELLITUS IN 19-YEAR- OLD SWISS MALES IN 27 YEARS

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Aims: In some parts of Europe type 1 diabetes seems to have increased during recent decades. To determine the prevalence of type 1 diabetes mellitus in 19-year-old Swiss males data covering the period 1972-1998 were analyzed.
Material and Methods: The cohort study included data from 9 surveys in 335'009 19-year-old Swiss males. At this age every Swiss male has to present himself to a compulsory examination of the Swiss army. All those with known type 1 diabetes are rejected without exception.
Results: A total of 470 subjects with type 1 diabetes were identified. The average prevalence rate for the nine birth cohorts was 1.40/1000 (95% CI 1.28, 1.54). Trend analysis showed no evidence of an increase in diabetes prevalence rates (overall χ^2=8.028, df=8, p=0.431; χ^2_{trend}=1.243, df=1, 0.25<p<0.5).
Conclusions: In Switzerland, contrary to reports from other European countries, analysis of army files covering a period of 27 years does not indicate that there is an increase in the prevalence of type 1 diabetes.

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SPACE-TIME CLUSTERING OF CHILDHOOD DIABETES AT DIAGNOSIS IN DEVON AND CORNWALL, ENGLAND

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Aim: To investigate whether there is evidence of space-time clustering in the onset of childhood Type 1 diabetes in Devon and Cornwall, England from 1975-96. Previous studies have shown conflicting results. Positive results may imply an effect of environmental factors in the aetiology of this disease. **Subjects and Methods:** All 522 newly diagnosed Type 1 diabetic children aged 0-15 years old from 1975 - 1996 contained in the population-based Cornwall and Plymouth Children's Diabetes Register were used in the analysis. The case ascertainment for this register was estimated 94.4% complete. Mantel's modification of Knox method was employed to calculate z statistics for each combination of space and time thresholds and a value of z greater than 1.645 or 2.326 indicates significance at 5% or 1% level (one-tailed). Time at diagnosis was defined as the date of first insulin injection and spatial referencing in the analysis was derived by patients' postcodes of residence at the time of diagnosis. The space and time thresholds were 1, 5, 10, 15, 20, 25, and 50 km and 28, 90, 360, 540, and 900 days, respectively. **Results:** Significantly greater numbers of observed close pairs than would be expected by chance have been found in the following combinations of critical cutoff values, 25 km and 360 days ($p < 0.05$), 50 km and 90 ($p < 0.05$), 540($p < 0.05$), and 900 days ($p < 0.05$) with the highest significance found in 50 km and 360 days ($p < 0.01$). **Conclusion:** There is evidence of space-time clustering in the onset of childhood Type 1 diabetes in the far south west of England. These results lend some support to the hypothesis that an external agent, such as an infection may be involved in the development of childhood onset diabetes.

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RISING INCIDENCE OF CHILDHOOD DIABETES IN YORKSHIRE, UK IS SEEN AT ALL AGES AND IN URBAN AND RURAL SETTINGS.

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Aim: To determine whether the incidence of childhood Type 1 diabetes in Yorkshire, UK continues to rise and in which age groups. Also to compare incidence trends in urban and rural settings.
Methods: 2205 children aged 0-14 years from the population-based Yorkshire Children's Diabetes Register diagnosed over a 20 year period (1978-97) were analysed. Using annual mid-year population estimates, sex-standardised incidence rates per 10^5/yr by age group (0-4, 5-9, 10-14) were tested by linear regression modelling. Districts (n=22) were classified as mainly urban or mainly rural by levels of person-based population density (<7 per hectare = rural, >7 per hectare = urban) and incidence trends tested.
Results: There was a significant rise in incidence overall and in each age group (0-14: 2.2%/yr $p<0.01$, 0-4: 2.1%/yr $p=0.03$, 5-9: 1.7%/yr $p=0.03$, 10-14: 3.1%/yr $p<0.01$). Urban incidence increased significantly from 11.7 per 10^5/yr to 18.7 ($p<0.01$) and rural incidence from 14.7 to 19.5 ($p=0.06$). On average, incidence in rural areas was 4.9 per 10^5 higher compared to urban areas ($p<0.01$). These differences were reflected in each age group. There was no evidence of epidemicity.
Conclusions: In Yorkshire, UK there has been a steady and significant increase in the incidence of Type 1 diabetes in children of all ages. This is seen in urban and rural populations. This contrasts with data from other areas where the increases in incidence were restricted to the under 5s.

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HOSPITALIZATION IN 6.007 CHILDREN AND ADOLESCENTS WITH TYPE 1-DIABETES AND IN THE GERMAN GENERAL POPULATION

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Reduction of diabetes-related hospitalization in children and adolescents is a major goal of structured diabetes care. Aim of this analysis was to estimate relative hospitalization risks and days in in a large cohort of pediatric patients with diabetes in Germany compared to the general German population of the same age.
Methods: Based on a prospective computer-based documentation program, established by the German Working Group on Quality Management in Childhood Diabetes, hospital admissions and days after diabetes onset in 1998 were ascertained. Hospital admissions and days in the general German population were derived from official hospital statistics. Age- and sex-specific and standardized incidence rates (IR) and days of hospitalization (standard: BRD population <25 years of age, categorized into 4 classes) were estimated in the diabetic cohort and and compared with the corresponding measures in the BRD population. In addition, standardized ratios of hospitalization incidence (SIR) and days ("SDR")of the diabetic cohort in comparison to the general population were calculated.
Results: 61 centers contributed 6,007 patients (52% male, mean age 12.8±4.4, mean diabetes duration 5.0±4.4 years, 4,349 years of follow-up in 1998). The estimated hospitalization IR's (* py^{-1}, 95%-CI) were 0.42 (0.40-0.44) and 0.1134 (0.1133-0.1136) in the diabetic and in the general population, respectively, and the hospital days (* py^{-1}, 95%-CI) were 2.73 (2.68-2.78 and 0.9678 (0.9675-0.9683). SIR and "SDR"(95%-CI) were 4.43 (4.23-4.64) and 3.37 (3.31-3.43), respectively. The ratios were higher in boys and in pubertal age.
Conclusions: Children and adolescents with diabetes had a 4.4 times higher hospitalization risk and 3.4 times more hospital days than the general population of the same age. These ratios were smaller as observed in Finland, Denmark, and Australia in the 1980'ies, probably due to time trends, different health care systems, or differences in diabetes care. A repetition of this study may evaluate interventions that aim at further reduction of diabetes-related hospitalization.

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POPULATION MIXING AND THE INCIDENCE OF CHILDHOOD ONSET TYPE 1 DIABETES IN YORKSHIRE, UK.

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Aims: Exposure to infections in early life decreases the risk of developing diabetes in animal models and in epidemiological studies. Areas with a high level of population mixing are likely to have high infectious load in the community. We used migration as a measure of population mixing to test the hypothesis that areas with high levels of population mobility have a lower incidence of childhood onset Type 1 diabetes.
Materials and Methods: Cases: 994 children (0-14 years) diagnosed with Type 1 diabetes between 1986 and 1994 from a population based register. Population data: the 1991 UK Census was used to calculate age/sex standardised incidence rates, a standardised deprivation score and person-based population density by census ward (n=532). A measure of population mixing based on information theory (the Shannon index) was calculated using detailed statistics on childhood migration patterns. Poisson regression was used to examine the effect of population mixing in children, adjusted for population density and deprivation score on diabetes incidence for all ages and 5 year age-groups (0-4,5-9 and 10-14 years).
Results: Effect of population mixing adjusted for deprivation and population density: 0-14 years - incidence rate ratio (IRR) 0.89, P=0.045, 95% confidence interval (CI) 0.79-0.99; 0-4 years - IRR 0.89, P=0.323 95%CI 0.71-1.12; 5-9 years - IRR 1.06, P=0.582, 95%CI 0.86-1.29; 10-14 years - IRR 0.80, P=0.007, 95%CI 0.68-0.94.
Conclusions: There is evidence that higher levels of childhood population mixing result in decreased incidence of Type 1 diabetes when adjusted for deprivation and population density and this effect is particularly strong in the 10-14 year age range.

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DO PARENTAL OCCUPATIONS INVOLVING SOCIAL MIXING AFFECT THE RISK OF CHILDHOOD DIABETES?

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Aims: To test the hypothesis that parents whose occupations involve high levels of social mixing, and therefore exposure to infections, reduces the risk of diabetes in their children. **Materials and Methods:** In a population-based case-control study the parents of 220 children (0-15 years) diagnosed with Type 1 diabetes between 1993-94, and 433 age/sex matched controls were interviewed. Mother's and father's occupations, recorded at the time of interview, were coded using the UK 1990 Occupational Classification. This lists 371 occupations 79 of which were classified as having potentially 'high' levels of social mixing (e.g. school teachers), 23 as 'medium' with the remainder classified as 'low'. This 'social mixing' classification was constructed independently by an occupational hygienist and has been used to assess the risk of childhood leukaemia among fathers whose jobs involve social mixing. Mothers' and fathers' occupations were grouped according to this classification. Due to small numbers in the medium exposure group (fathers: 8 cases, 13 controls; mothers: 10 cases, 17 controls), low and medium exposure levels were combined. Associations between estimated level of parental social mixing (high vs. low + medium), age at diagnosis (0-15, 0-4, 5-15) and offspring's risk of diabetes were assessed using odds ratios (OR) produced using conditional logistic regression. **Results:** For all ages (0-15 years), no associations were observed between high parental occupational social mixing and childhood diabetes (fathers: OR 1.18; 95% CI: 0.78-1.77, based on 44 exposed cases; mothers: OR 1.08; 95% CI: 0.77-1.51, based on 89 exposed cases). A reduced risk for high maternal occupational social mixing among children aged 0 to 4 was observed (OR 0.74; 95% CI: 0.34-1.61, based on 12 exposed cases). **Conclusions:** These findings do not suggest that childhood diabetes is related to the level of social mixing that parents experience at work. However, the protective effect seen for high levels of maternal occupational social mixing among children aged 0 to 4 years requires further investigation.

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IS CHILDHOOD DIABETES A ZOONOSIS?

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Aims: The incidence of Type 1 childhood onset diabetes is higher in sparsely populated rural areas and we investigated whether contact with farm and domestic animals might result in an increase in the risk of cross-species transmission of infective agents which initiate islet cell damage.

Materials and Methods: This study examined the relationship between contact with animals and onset of diabetes in a case-control study with 220 incident cases and 433 age/sex matched controls derived from a population-based register of children with Type 1 diabetes (0-15yrs). Parameters used were presence of a pet (farm animals, mammals, birds) before and after birth Odds ratios (OR) with exact 95% confidence intervals (CI) were calculated using conditional logistic regression.

Results: Contact with animals occurred in 63% of cases and 58% of controls during mothers' pregnancy and 80% of cases, 82% of controls after birth. The proportion of animal types were similar in both groups. Four controls and no cases had diabetic pets. No significantly raised or lowered OR were found for contact with animals.

Exposure	cases (%)		controls(%)		OR	95% CI	
Before birth							
Any pet	138	63	250	58	1.22	0.88	1.70
Farm	5	3	8	2	1.27	0.40	4.10
Mammals	132	60	238	55	1.22	0.88	1.69
Birds	10	5	19	4	1.00	0.45	2.22
After birth							
Any pet	175	80	356	82	0.83	0.55	1.25
Farm	10	5	13	3.0	1.52	0.65	3.56
Mammals	168	77	340	79	0.87	0.60	1.28
Birds	32	15	51	12	1.30	0.79	2.14

Conclusions: These data do not support the concept that childhood diabetes is a zoonosis. Infective agents which initiate islet cell damage in genetically susceptible individuals may rely on intra rather than inter-species transmission.

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β-CASEIN A1 CONSUMPTION AND INCIDENCE OF TYPE 1 DIABETES IN GERMANY

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Aims: Several observations have linked the global intake of cow milk to the incidence of type 1 diabetes. A recent study has further demonstrated an association between the intake of the β-casein A1 variant and the incidence of the disease in several countries. Our study was performed to investigate a possible relationship between the consumption of this β-casein variant and the incidence of type 1 diabetes within the different states of Germany.

Methods: The mean consumption of β-casein A1 was estimated by using the dairy breeds distribution in the German states, the mean allele frequency of β-casein A1 in the breeds and the mean intake of cow milk in Germany. The resulting values were correlated to the local incidence of type 1 diabetes within the different states of Germany.

Results: In Germany, there are substantial differences with regard to the regional consumption of β-casein A1. Similarly, the incidence of type 1 diabetes in children under the age of 5 shows considerable variation. Upon correlation, there is a significant association between these two variables ($p < 0.05$, Poisson regression) within Germany.

Conclusions: Regional differences in the mean intake of β-casein A1 may contribute to regional variation of the incidence of type 1 diabetes. Our study warrants further investigation of a possible role of β-casein variants in the pathogenesis of type 1 diabetes.

(* equal contribution)

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PREVALENCE OF AUTOANTIBODIES TO PANCREATIC βCELL ANTIGENS IN SARDINIAN WOMEN WITH GESTATIONAL DIABETES MELLITUS

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Aims: The aim of the study is to establish the prevalence of autoantibodies (AA) to pancreatic βcell antigens in women with Gestational Diabetes Mellitus (GDM) belonging to Sardinian Region, which share with Finland the highest incidence of type 1 Diabetes in the world, and its correlation with the risk of developing diabetes later in life.

Materials and Methods: We have investigated 109 women with GDM (mean age 32.7 years; range 20.5-42 years) and the same number as control population.

We have already tested 87 GDM for ICA (Islet Cell Autoantibodies), 66 for GADA (Glutamic Acid Decarboxylase Autoantibodies) and for IA2A (Tyrosine Phosphatase Autoantibodies). GDM was diagnosed between the 24th and 28th week of gestation with OGTT (Oral Glucose Tolerance Test - 100g glucose load).

ICA were detected by indirect IFL, GADA and IA2A were detected with radioimmunoassay (^{125}I-labelled human recombinant). HLA analysis was performed by sequence-specific oligonucleotide typing.

Results: In GDM-women, the prevalence of ICA was 3.4%, of GADA was 7.5%, and of IA2A was 1.5%. 8 GDM women were positive for almost one AA and after delivery 5 of them developed Diabetes. The other three have not developed diabetes so far (OGTT neg. in follow up) but are still positive for AA.

31 women with previous GDM attended the follow up with OGTT by 9 months after delivery: 25.8% developed glucose tolerance abnormalities. Regarding to the frequencies of HLA-DRB1*03 DRB1*04, 73% of GDM-women were positive for DR3 or DR4 (16-22).

Conclusions: AA and HLA typing are useful markers to predict the development of diabetes in women with previous GDM especially in a region with the highest incidence of diabetes.

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THE PREVALENCE OF GADA AND IA-2A ANTIBODIES IN WOMEN WITH GESTATIONAL DIABETES TREATED WITH DIET.

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Gestational diabetes is considered an important factor complicating foetal development. Furthermore it might potentate the risk of type I maternal diabetes after delivery. Nowadays, on the basis of a detection of antibodies against islet cells (ICA, GADA, IA-2A, IAA), we can recognize autoimmune disorders typical for the type I diabetes. **Aims:** Therefore, the aim of our study was to estimate the prevalence of islet cells autoantibodies (Abs) in women with a history of gestational diabetes and to assess, if they might be a risk factor for foetus development and gestation outcome. **Material and methods:** Our investigations were carried out in 156 patients with the history of gestational diabetes (treated with diet), 6 weeks after delivery. GADA, IA-2A, HbA1c and lipid profiles were estimated. Then IVGTT was performed to measure the first phase of insulin secretion. The number of previous abortions per number of pregnancies and birth weight of children were also assessed. **Results:** In the studied population GADA were detected in 7.0% and IA-2A in 3.2%. The prevalence of Abs was higher than in the healthy population but lower than observed among women with family history of type I diabetes. The presence of 2 types of antibodies was found in 3.8% of patients. In the group with the autoimmune disorders, significantly higher birth weights and more frequent failures of previous pregnancies were found. **In conclusion:** the foregoing data suggest that the detection of antibodies against the beta cells in women with gestational diabetes might be a serious risk factor and a possible indication for early insulin treatment. As a result the better prognosis of gestation outcome is expected. However, we believe that the further prospective studies are required to verify our statement.

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RISK OF CHILDHOOD DIABETES RISES WITH MATERNAL AGE AT DELIVERY

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Aims: To examine the influence of maternal and paternal age at birth upon the risk of diabetes in the offspring. **Methods:** A population-based cohort of families with a child with type 1 diabetes was studied using survival analysis. **Results:** Of 2915 offspring included in the analysis, 1298 have diabetes (median age at diagnosis 10.6 yr) and 1617 remain non-diabetic at median age 16.9 years (range 0.6 - 43). Both maternal and paternal age were related to risk in the offspring ($p<0.0001$) while birth order was not ($p=0.54$). By age 20, the cumulative risk of diabetes was 45% (95% CI 41-49) in children of mothers in the youngest quintile for birth age (median 21 yr) as against 68% (63-74) in the oldest quintile (median 34 yr) with risks of 48%, 56% and 56% for the intermediate quintiles. Paternal age at delivery had a similar effect, with risk ranging from 46% (42-51) in the youngest quintile to 62% (56-67) in the oldest. Cox regression analysis showed that maternal age and offspring gender were independent determinants of risk but paternal age was not. There was a small but significant inverse correlation between age at diagnosis and maternal age at birth (R^2 0.2, $p<0.0001$). **Conclusions:** Risk of diabetes increases markedly with maternal age at delivery throughout reproductive life, and rising maternal age is associated with a minor decrease in age of diagnosis. This study in children with similar genetic susceptibility provides further evidence that prenatal factors have important effects upon the risk of diabetes development in the child. Maternal age at delivery has risen in the UK over the last two decades, and this may have contributed to the increase in incidence of diabetes in young children.

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NON-AUTOIMMUNE FULMINANT TYPE 1 DIABETES: A NOVEL SUBTYPE OF TYPE 1 DIABETES

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Aims: This study was conducted to clarify the clinical and pathological characteristics of idiopathic or non-autoimmune type 1 diabetes. **Material and Methods:** Consecutive 60 Japanese adult type 1 patients were reviewed at the onset of overt diabetes after divided to 2 groups according to presence (AI group) or absence (IP group) of glutamic acid decarboxylase antibodies. **Results**: Thirty-six patients were classified to AI group and 20 patients to IP group. IP group could be further divided into low HbA_{1c} group (IP-L, HbA_{1c} <8.5%, 12 patients) and high HbA_{1c} group (IP-H, HbA_{1c} >11.5%, 8 patients). Mean plasma glucose value was significantly higher in IP-L group (40.5 mmol/l) than in AI group (22.3 mmol/l; $p<0.0001$) and IP-H group (24.2 mmol/l; $p=0.0257$) in spite of lower HbA_{1c} level in IP-L group. Mean duration with hyperglycaemic symptoms of IP-L group was only 4.0 days and significantly shorter than that of AI group (53.6 days; $p<0.0001$) or IP-H group (35.5 days; $p=0.0002$). Diabetic ketoacidosis was observed in all IP-L group patients but only in 18.2% in AI group ($p=0.0003$) and in 40% in IP-H group ($p=0.0275$). Mean urinary C-peptide excretion was significantly lower in IP-L group (3.1 μg/day) than in AI group (21.9 μg/day; $p<0.0001$) and IP-H group (22.8 μg/day; $p=0.0004$). Serum total amylase or elastase-1 level was elevated in all IP-L group patients but not any in other 2 groups. Immunohistological examination has revealed neither insulitis nor MHC class I hyper-expression in islets but T-lymphocyte-predominant infiltrates in exocrine pancreas in all 3 patients with similar characteristics to IP-L group. **Conclusions**: Within idiopathic or non-autoimmune type 1 diabetes, there exists a novel subtype, "non-autoimmune fulminant" type 1 diabetes, which is characterized by the absence of insulitis and islet autoantibodies, remarkably abrupt onset and elevated serum pancreatic enzyme levels.

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Increased transmission of Intercellular Adhesion Molecule-1 469E allele in type 1 Romanian diabetic families.

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Type 1 diabetes is an autoimmune pathogeny disease conditioned by genetic factors. Recent studies (mainly on NOD mice) show the importance of Intercellular Adhesion Molecule-1 (ICAM-1-CD54) in the autoimmune destruction of pancreatic β cells and the possibility to delay or block the insulitis with anti ICAM-1 mAb. There are several polymorphisms of ICAM-1 gene (chromosome 19p13.3-2) in humans. We typed two of these polymorphisms: the 657A/G substitution in exon 4 (241G/R polymorphism in the AA sequence) and the 1548A/G substitution in exon 6 (469E/K polymorphism in the AA chain) - in 204 Romanian diabetic families (756 individuals); 212 type 1 diabetic probands (106M/106F) with onset of disease between 9 months and 43 yr; median age at onset 12.1±6.7 yr. and 544 non-affected parents and siblings. The typing was made by SSP-PCR and electrophoresis of PCR products on ethimidium bromide stained agarose gels. We studied the transmission of ICAM-1 alleles to affected and non-affected siblings using the Transmission Disequilibrium Test (TDT). We found a not significant increased transmission of 241R allele to diabetics (37 T/22 NT, 62.71% transmission, p-0.034) compared with the non-affected siblings (23 T/20 NT, 53.49% transmission, p-0.38). However the transmission of 469E allele was significantly increased to the affected siblings (122 T/70 NT, 63.54% transmission, p-0.00011) compared with the non-affected (68 T/61 NT, 52.71% transmission, p-0.31). We found a 10% difference between the frequency of ICAM-1 469E/E homozygotes in affected versus non-affected (29.25%/20.40%). The transmission of haplotypes in the affected show a protective haplotype 241G/469K, 32.39% transmission, p-2.75e-05 and a susceptibility haplotype 241G/469E, 63.57% transmission, p-0.0013. The transmission of these haplotypes was aprox 50% to the non-affected. **Conclusions:** We found a significantly increased transmission of ICAM-1 469E allele in diabetics compared with their non-affected siblings. Correlated with the experimental data from NOD mice, this supports the possible significant role of ICAM-1 in the ethiopathogeny of type 1 diabetes.

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NO LINKAGE OF POLYMORPHISMS IN THE HUMAN FAS GENE WITH IDDM

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The human Fas gene, localized on chromosome 10q24.1 encoding a cell surface receptor involved in the induction of programmed cell death or apoptosis was evaluated as a candidate susceptibility gene for IDDM. Fas mediated apoptosis plays a major role in maintaining peripheral self tolerance and in down-regulating an immune response. Mutations in the human Fas gene cause Auto-immune Lymphoproliferative Syndrome. There is evidence that Fas mediated apoptosis plays a role in autoimmune β-cell destruction.
We have completed a molecular scanning of the entire coding region of the human Fas gene (exons I -IX, including exon - intron borders and the 3'UTR) utilizing SSCP and heteroduplex analysis.
We identified eight mutations , of which two have been previously described. There were six single base substitutions, one single base insertion and one single base deletion. Two of the mutations had a high heterozygosity index i) a G to T substitution in intron V leading to a new Bfa I RFLP and ii) a silent C to T substitution in codon 198 leading to a Dra I RFLP. These were tested for association with IDDM in 96 unrelated IDDM subjects and 76 healthy controls. There were no evidence for association. A Danish family material comprising 144 IDDM sib pair families, (302 affected offspring and 137 unaffected offspring) and 103 parent-offspring IDDM multiplex families (103 affected and 112 unaffected offspring) were typed for the Dra I RFLP, and the data were analyzed utilizing the transmission disequilibrium test, TDT. There was no evidence for linkage to IDDM, P_{TDT} 0.84.
In conclusion, there was no evidence of neither association nor linkage of the Fas gene polymorphisms to IDDM.

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INVESTIGATION OF INTERFERON-γ AND INTERLEUKIN-4 GENE POLYMORPHISM IN TYPE 1 DIABETES

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Recent studies have shown that genes outside of the HLA region are involved in determining susceptibility to type 1 diabetes. Polymorphisms in the coding and non-coding regions of the genes encoding cytokines may be involved in modulating the immune response to self and non-self antigens. There is increasing evidence that the imbalance between Th1 and Th2 lymphocyte subsets plays a key role in the development of experimental and clinical type 1 diabetes. The aim of this study was to investigate the frequency of CA dinucleotide repeat polymorphisms in the interferon-γ (IFNG) gene and C→T substitute at position of -590 of the intereukin-4 (IL-4) gene in 236 Caucasoid patients with type 1 diabetes. The IFNG region was amplified using the polymerase chain reaction (PCR) with specific amplimer (the sense amplimer was labelled with P^{32}-ATP) and the product separated using polyacrylamide gel electrophoresis. Meanwhile, the IL-4 promoter polymorphism (-590 C→T) was analysed in our population by specific PCR amplification and BsmFI restriction endonucease digestion. There was a highly significant increase of the 122/122 IFNG genotype in the patients compared to normal healthy controls (56.0% vs 13.0% $p<0.0001$) as well as a significant increase in the 122 IFNG allele in the patients compared to controls (0.51 vs 0.31 $p<0.000001$). No differences were found with age of onset of diabetes or gender. In contrast, no differences were found in the frequency of the -590 polymorphism of IL-4 gene between the patients and controls. These results suggest that polymorphisms of the INFG gene (Th1) may modify the function of this pro-inflammatory mediator and the cytotoxic response to pancreatic islet β-cells.

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THE NAD(P)H:QUINONE OXIDOREDUCTASE P187S POLYMORPHISM: LINKAGE ANALYSIS IN DANISH TYPE 1 DIABETES FAMILIES.

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Recent genome screenings have identified novel regions of interest for susceptibility to type 1 diabetes. One of these is a 30-35 cM region mapping to chromosome 16q22-q24 (*D16S515-D16S520*), where also the gene encoding NAD(P)H:quinone oxidoreductase (*NQO1*) maps. Association of a C to T transition gene polymorphism at position 609 of the *NQO1*, resulting in a proline to serine substitution at amino acid position 187 (P187S) in NQO1 with type 1 diabetes was suggested in a Japanese IDDM population. NQO1 is involved in protection against oxidative stress which is likely to be involved in β-cell destruction and the P187S mutation results in reduced NQO1 activity. Hence, the aim of the present study was to investigate if the NQO1 P187S polymorphism was linked to type 1 diabetes in Danish patients by use of the transmission disequilibrium test (TDT). Using a PCR based RFLP assay we genotyped a population-based sample of 247 Danish nuclear type 1 diabetic families (454 parents, 405 affected and 249 unaffected offspring) for the NQO1 P187S polymorphism. The frequency of the S187-encoding allele was 17% in the parental generation. It was possible to evaluate transmissions from 125 heterozygous parents to 169 affected and 119 unaffected offspring. Random transmission patterns were observed to all affected offspring (51% T (mutant) alleles transmitted, P_{tdt} = 0.82), to index cases, defined as the first offspring in each family to have type 1 diabetes (51% T alleles transmitted, P_{tdt} = 0.77) as well as to unaffected offspring (50% T alleles transmitted, P_{tdt} = 0.93). Conditioning for *IDDM1*/MHC by stratification of the affected offspring into high and non-high IDDM HLA risk groups did not reveal of linkage in any of the groups (P_{tdt} > 0.41).
In conclusion, we were not able to demonstrate linkage between the P187S NQO1 polymorphism and IDDM in the Danish IDDM material. Hence, the *NQO1* polymorphism is not likely to be an etiological mutation underlying the reported linkage of the 16q22-q24 region.

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TELOMERE LENGTH, TYPE 1 DIABETES AND IMMUNE MARKERS IN THE DANISH DIABETIC TWIN STUDY

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Aims: To examine (1) whether the length of telomeres, i.e. the ends of chromosomes, are shorter in white blood cell (WBC's) from twins with Type 1 diabetes than in healthy twins. (2) whether there is a correlation between telomere length and diabetes related immune markers. (3) whether the underlying reason for a possible positive finding, could be that diabetic subjects inherit relatively short telomeres or that telomere length in patients reflects an increased turn-over rate in WBC's engaged in the destruction of the beta-cells.

Materials and methods: Study subjects were Danish monozygotic and dizygotic twin pairs concordant and discordant for Type 1 diabetes and recruited from the population based Danish Twin Register. Telomere length in WBC's and GAD65 antibodies, IA-2 antibodies and islet cell antibodies were measured in blood samples from the twins. Data were analysed by means of (1) a linear model with variance component terms and (2) Spearman's correlation and sum-difference plots.

Results: DNA from a total of 52 pairs of twins, 18 monozygotic and 34 dizygotic pairs, were available. The telomere length of WBC's of twins with Type 1 diabetes were NOT different from that of non-diabetic twins. There was no significant correlation between telomere length and levels of Gad antibodies, IA-2 antibodies or islet cell antibodies. Furthermore, the sum-difference plot did not reveal any obvious patterns in the distribution of mean of telomere length versus difference in antibody level in the pairs.

Conclusions: In this study there was no relationship between telomere length and diabetic status. Furthermore, there was no correlation between telomere length and levels of GAD antibodies, IA-2 antbodies or islet cell antibodies. Thus, in Danish twins telomere length does not seem to reflect a changed turn-over rate due to the immune mediated destruction of the beta-cells.

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AMINO ACID VARIANTS OF THE VITAMIN D-BINDING PROTEIN ARE NOT ASSOCIATED WITH TYPE 1 DIABETES IN CAUCASIANS.

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Aims: No gene for type 1 diabetes mellitus has been identified so far. There is evidence that vitamin D has immunomodulatory properties and can prevent the development of insulitis in an animal model. Vitamin D-binding protein (DBP) is involved in the functioning of this hormone. Thus this gene may be regarded a candidate for type 1 diabetes. There are two known polymorphisms in exon 11 of DBP that result in amino acid variants: at codons 416 GAT→GAG (Asp→Glu) and 420 ACG→AAG (Thr→Lys). These changes generate HaeIII and StyI restriction sites, respectively. The aim of our study was to examine the association of the alleles, genotypes, and haplotypes at these codons of DBP with type 1 diabetes. **Materials and Methods:** The study group consisted of 181 unrelated patients with type 1 diabetes (mean age at diagnosis: 10.9 yrs, mean age at examination: 36.2 yrs) and 172 healthy controls (mean age at examination: 55.5 yrs), all of them Caucasians. Exon 11 was amplified by polymerase chain reaction followed by digestion of PCR product. The distribution of variants in the group of the diabetic patients and the controls were compared by $\chi 2$. **Results:** At both residues the distribution of the alleles was similar ($p=0.80$ and $p=0.35$, respectively). The frequencies of the genotypes are shown below:

Codon 416	Asp/Asp	Asp/Glu	Glu/Glu	P=0.34
Type 1 patients	41 (22.7%)	80 (44.2%)	60 (33.1%)	
Controls	32 (19.6%)	85 (52.2%)	46 (28.2%)	
Codon 420	Thr/Thr	Thr/Lys	Lys/Lys	p=0.35
Type 1 patients	95 (52.5%)	76 (42.0%)	10 (5.5%)	
Controls	87 (50.3%)	65 (39.9%)	16 (9.8%)	

The distribution of four haplotypes defined by the two loci was also similar in both groups ($p=0.32$). **Conclusions:** Our results do not show the association of DBP variants with type 1 diabetes mellitus in Caucasians.

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PROMOTER SEQUENCE STUDIES OF THE *LGALS3* (GALECTIN-3) GENE - AN IDDM SUSCEPTIBILITY GENE.

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Galectin-3 is a β-galactoside-specific lectin found in many species and cell types. The gene is located on the long arm of chromosome 14 (14q21). Results from genome-wide screenings and partial ones have showed some evidence for linkage in the Galectin-3 gene region, *D14S276*. In the present study we have screened for polymorphism's in the promoter region and the intron 2 of the Galectin-3 gene which also has showed promoter activity. Combining SSCP (Single Stranded Conformation Polymorphism) and cycle sequencing methods we have identified 3 polymorphism's within 60 bp of the promoter region. Two of the polymorphism's are single base substitutions leading to different restrictions sites (*MseI* and *StuI*). The third polymorphism is a 12 bp deletion which also lead to a different restriction sites (*Eco0109I*). All three polymorphism's have high heterozygosity and were analysed for linkage to IDDM in a large homogeneous Danish family material comprising 251 families (1041 individuals) with 410 IDDM-affected offspring. Transmissions of wildtype (W) and mutant (M) alleles from heterozygous parents to offspring are shown in the table below:

	IDDM		*non-IDDM*	
	W	*M*	*W*	*M*
MseI (-715)	154	140	105	98
Eco0109I (-698)	160	157	100	114
StuI (-659)	106	110	56	66

The numbers in brackets are the distance from the transcription site. Even though Galectin-3 is a very promising candidate gene for IDDM we found no linkage to IDDM of the identified polymorphism's. An etiological mutation may still be found in the coding region of the gene, the 3'UTR or when combining variations in the promoter region with variations in the coding region as specific haplotypes.

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ASSOCIATION OF THE 5' INSULIN GENE POLYMORPHISM IN SPANISH TYPE 1 DIABETES: HETEROGENEITY WITH BASQUES

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Introduction: The susceptibility locus for Type 1 diabetes (*IDDM2*) has been identified as allelic variation at the VNTR (*Variable Number of Tandem Repeats*) region upstream the human insulin gene (11p15.5). Short alleles (class I) confer risk to Type 1 diabetes, while long alleles (class III) are dominantly protective. Our previous results confirm this association in Basques*.

Objective: To analyse the contribution of the *IDDM2* locus to Type 1 diabetes risk in Spanish population.

Patients and Methods: We studied 67 Spanish families with Type 1 diabetes (71 patients and 129 first-degree relatives). VNTR typing was performed by high-resolution electrophoresis of fluorescent PCR amplifications (subtyping of class I alleles) or silver staining of polyacrilamide gels (class I/class III). For association analysis, alleles were categorised as *diabetic* or *non-diabetic* using an AFBAC (*Affected Family-Based Controls*) approach.

Results: We obtained 267 alleles whose frequencies were as follows:

	SPANISH ALLELES		**BASQUE ALLELES***	
Alleles	**Diabetics (138)**	**Non-Diabetics (129)**	**Diabetics (147)**	**Non-Diabetics (125)**
CLASS I	0.89	0.77	0,857	0,600
CLASS III	0.11	0.23	0,143	0,400
	p = 0,01		**p = $1,5 \cdot 10^{-6}$**	

Seventeen different Class I alleles (626-858 u) were identified in Spanish people with no significant differences in the conferring risk to Type 1 diabetes.

Conclusions: 1/ Insulin VNTR Class I alleles confer risk to Type 1 diabetes in Spanish population. 2/ Class I/Class III VNTR-INS allelic distribution between Spanish and Basques is different. 3/ The non diabetogenic effect of some Class I subtypes (*allele 814*) maybe masked by the high frequency of the Class I alleles in Spanish population.

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ASSESSMENT OF THE INTERACTION BETWEEN HLA-DQB1 ALLELES AND GAD65 IN A STUDY OF CASES AND PARENTS IN CHILE

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Aim: The purpose of this study is to estimate whether the association between DQB1*0201 and DQB1*0302 alleles with childhood diabetes, depends on the presence of glutamic acid decarboxilase (GAD65) autoantibodies. **Material and Methods**: A study of incident cases and parents was carried out in Santiago, Chile during 1997-98. In this design, each new case is matched with three pseudosibs controls constructed with combinations of parental alleles. The use of the case-parental design eliminates the possibility that case-controls differences are due to selection of controls whose genetic backgrounds differ systematically from those of cases. HLA-DQ polymorphism was determined in cases and parents from n = 88 families using polymerase chain reaction and oligonucleotide dot-blot analysis. Detection of GAD abs in the 88 incident cases was done using a simple radio-binding asssay. **Results:** HLA-DQ polymorphism is strongly associated with type 1 diabetes, with crude odds ratios of 3.3 (95% Confidence Interval: 1.7-6.4) for DQB1*0201 allele and 9.8 (95%C.I: 4.9-19.6) for DQB1*0302 allele. When these HLA-disease associations were assessed in the subset of families with GAD65+ cases, the odds ratios were estimated as 18 (95%C.I: 2.4 – 134) for DQB1*0201 allele, and 5.4 (95%C.I: 2.4 – 12.1) for DQB1*0302 allele. In families with GAD65- cases, the odds ratios were calculated as 1.8 (95%C.I: 0.7-4.2) for DQB1*0201 allele, and 38.5 (95%C.I: 5.3-283) for DQB1*0302 allele. There is evidence of statistical interaction between GAD65 and DQB1*0201 allele (p = 0.01) or DQB1*0302 allele (p = 0.03). **Conclusion:** HLA class II alleles DQB1*0302 and DQB1*0201 display distinc associations with type 1 diabetes depending on the autoimmunity to GAD65. (supported by Fondecyt 1970204 and SAF 97-0251).

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DOUBLE BLIND TRIAL OF ORAL INSULIN IN RECENT ONSET TYPE 1 DIABETES

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Induction of immunotolerance to insulin can be obtained in animal models of Type 1 diabetes by oral administration of insulin. In the DPT1 trial in the U.S. oral insulin is tested to prevent the disease insurgence. If given in the early stages of Type 1 diabetes, oral insulin can protect residual beta cell functions and possibly induce disease remission. **Aims:** The present study was designed as a double blind trial in recent onset Type 1 diabetes patients (mean age 14 years ± 8 SD) in whom oral insulin (5mg daily) or placebo were administered for 12 months in addition to intensive subcutaneous insulin therapy. **Materials and Methods:** A total of 78 patients with Type 1 diabetes (< 4 weeks duration) were included in the trial. Basal C-peptide, glycated hemoglobin and insulin doses were monitored throughout the study. **Results:** Overall and without distinction between age at diagnosis, mean C-peptide secretion in oral insulin treated patients ranged from 0.96 ng/ml at diagnosis to 0.98 ng/ml (3 months), 1.07 ng/ml (6 months), 0.83 ng/ml (9 months), and 0.73 ng/ml (12 months) after diagnosis. In the placebo group, basal C-peptide levels were 0.84 (diagnosis), 1.10 (3 months), 1.04 (6 months), 0.72 (9 months) and 0.53 (12 months). Insulin requirement was similar between the two groups and mean HbAlc levels were 7.2% ± 1.5 in the oral insulin group and 7.4% ± 1.2 in the placebo group (NS). **Conclusion:** Results of this study indicate that the addition of 5 mg of oral insulin does not overall modify the course of the disease in the first year after diagnosis

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IgG SUBCLASS RESPONSES TO INSULIN, GAD, AND IA-2 AFTER INSULIN EXPOSURE IN PRE- AND NEW-ONSET DIABETES.

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Aims: Insulin immunization in animal models induces T helper 2-like antibody subclass responses to insulin and other β cell antigens and protects from progression to overt diabetes. The aim of this study was to determine whether exposure to insulin in man resulted in a similar subclass bias of the humoral response. **Methods:** IgG subclass antibodies to insulin (IA), GAD, and IA-2 were measured at 3-month intervals after treatment with insulin in 25 patients with newly diagnosed type 1 diabetes treated with intravenous (iv) plus subcutaneous (sc) or sc insulin alone from diagnosis with follow-up 1-3 yrs post onset, 5 newly diagnosed patients treated with Cyclosporin A (CsA) and insulin for 12 months, and 7 antibody positive relatives treated with iv plus sc insulin (SIP trial). RBAs incorporating IgG subclass-specific sepharose were used to measure antigen specific subclasses. **Results:** IgG1- and IgG4- IA were the dominant responses induced in most subjects. IgG1-IA were the first detected and were maximal at 6-9 months after treatment. IgG4-IA appeared later, but at 12 months were often the dominant subclass. Responses were higher and reached maximum levels earlier in children compared to adults, and no differences in subclass profile were found with respect to the route of insulin administration. Insulin prophylaxis in relatives gave a similar profile with marked decline in IgG1-IA after cessation of daily sc insulin. CsA treatment caused supression of IA responses and rebounding high titre IgG1-IA after its removal. Despite the presence of IgG4-IA in most subjects, a shift to IgG4- anti-GAD or IA-2 was not found up to 3 years after start of therapy. **Conclusions:** IgG4-IA following insulin therapy in subjects with newly diagnosed or pre-type 1 diabetes may reflect induction of Th2 immunity, but there is no evidence of subsequent spreading of potentially Th2 associated IgG4 responses to other autoantigens.

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GAD, IA-2 AND INSULIN AUTOANTIBODIES IN RELATION TO SOME RISK FACTORS IN SIBLINGS OF EGYPTIAN CHILDREN WITH TYPE 1 DIABETES

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The **Aim** of the present work has been to screen siblings of children with type 1 diabetes for some immunological markers of the disease, namely GAD, IA-2 and Insulin autoantibodies and to study their possible correlation with other risk factors. **Material** included siblings of 151 child with type 1 diabetes (287 healthy siblings) besides 100 healthy control subjects matched for age and sex. **Methods**: All subjects were subjected to estimation of GAD antibodies using radioligand binding assay developed by Peterson et al in 1994, IA-2 by radioligand assay and Insulin autoantibodies (IAA) using ELISA technique. Subjects with positive immunological markers were screened for HLA-DR3 and DR4 and first phase insulin release (FPIR) after IV glucose infusion. **Results** revealed that 39.7% of healthy siblings were positive for one or more autoantibodies, 22.8% for 2 antibodies and 4.38% for 3 antibodies. GAD antibodies were present in 27.9%, IA-2 antibodies in 9% and IAA in 15.3% of healthy siblings. These prevalences were statistically significantly higher than those found in control subjects: 7%, 2% & 1.5% respectively. No relation could be elicited between positivity of immunological markers in the sibling and his/her age, sex, duration of breast feeding, age at cow's milk feeding nor with parents consanguinity. On the other hand, HLA-DR3, HLA-DR4 and DR3/DR4 were found in 29.4%, 58.8% & 82.3% respectively in seropositive subjects while in 27.5%, 32.5% & 45% in seronegative healthy siblings. FPIR less than 48 µU/ml was found in 52.9% of seropositive siblings. **Conclusion**: The high prevalence of positivity of autoantibodies among siblings of type 1 diabetic children suggests the importance of an environmental factor, perhaps via a mechanism of antigenic mimicry. Also the results in relation to other risk factors may help to a better understanding of the immuno-genetic susceptibility and/or pathogenesis of type 1 diabetes in a particular population.

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Improved risk prediction for IDDM by testing for the combined presence of HLA-DRb1Lys71+/+ genotype and GAA and ICA512/IA-2 (ICA) autoantibodies

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Insulin-dependent diabetes mellitus (IDDM) is an autoimmune disease characterized by destruction of islet ß cells, presumably by auto-reactive T-cells recognizing critical self-antigens presented by particular HLA alleles. Several autoantibodies including autoantibodies to glutamic acid decarboxylase (GAD65) and Islet cell antigen ICA512/IA-2 have been identified. Recently, at the DNA level, we have found that alleles encoding a lysine at position 71 of the DRb1 chain (DRb1Lys71+) very strongly associated with IDDM and that DQb1Asp57- had an additive effect on DRb1Lys71+ in the development of IDDM [1]; this was confirmed by haplotype analysis of HLA genes in Danish multiplex IDDM families [2]. In the present study we have genotyped eighty-one Danish multiplex families (374 individuals) with 165 IDDM affected sibs for HLA-DRB and DQ genes at the genomic level by PCR and sequence specific oligonucleotides (SSO's), and we also tested for the presence of GAD65 and ICA512/IA-2 autoantibodies. The predictive value of tests were calculated by Prevalence corrected Positive and Negative Predictive Value (PcPPV, PcNPV) formulas (Zamani et al. 1998). The **aim** of this study was to investigate whether a correlation existed between HLA susceptible genotypes for IDDM, particularly DRb1Lys71+/+, and the presence of these autoantibodies. Furthermore the predictive value of combination of DRb1Lys71+/+ genotype, GAD65 and ICA512/IA-2 autoantigens was examined to determine whether their combination improves prediction of IDDM. The results showed that DRb1Lys71+/+ associated significantly with the presence of GAD65 autoantibody (GAA+) (0.00017, RR=24.55) while DQB1*0302+/x, DRb1Lys71+/+ and DRB1*0401+/+ (encodes Lys71) were associated with ICA512/IA-2 autoantibody (ICA+). No association was found between DQb1Asp57-/- and GAA+ or ICA+ autoantibodies. These **results** significantly increase the predictive value of testing for IDDM. While the PcPPV for DRb1Lys71+/+ genotype was 0.3227 in first degree relatives, which means that by testing for this genotype, a 32.27% risk for IDDM can be predicted (prenatal and postnatal diagnosis). When DRb1Lys71+/+ is combined with GAA+, the PcPPV increased to 0.6145 and PcPPV for GAA+/ICA+ was 0.7175 in first degree relatives.

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EFFECTS OF A PROTECTIVE DIET ON PEYER'S PATCH, MESENTERIC LYMPH NODE AND ISLETS CELLS FROM BB RATS

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Aims: The effects of substituting a plant-based control diabetogenic diet (NIH diet) by a protective hydrolyzed casein diet (HC diet) upon selected metabolic and functional variables were investigated in Peyer's patch cells, mesenteric and pancreatic lymph node cells and pancreatic islets from 50-d old control (BBc) or diabetes-prone (BBdp) BB rats. **Methods:** The plasma D-glucose and insulin concentrations, the protein and insulin content of pancreatic islets, the metabolism of D-glucose and its insulinotropic action in islets first cultured for 24 h in the absence or presence of IL-1ß, the production of IFN-γ and IL-10 by mesenteric lymph node cells cultured for 48 h in the absence or presence of concanavalin A, the mitogenic activity of Peyer's patch cells and pancreatic lymph node cells in the absence or presence of the same lectin and the biosynthetic activity of Peyer's patch cells were measured in the BBc and BBdp rats fed either the NIH or HC diet. **Results:** Two major novel informations emerged from this study. First, in immune cells, diet HC increased to a greater extent the responsiveness to concanavalin A of certain metabolic and functional variables in BBdp rats than in BBc rats. Second, pancreatic islet cells of BBdp rats were less sensitive to IL-1ß than those of BBc rats and this difference was further accentuated when the animals were fed the HC, rather than NIH, diet. **Conclusions:** These findings afford further support to the view that, in BB rats, changes in the biological behaviour of Peyer's patch cells, mesenteric and pancreatic lymph node cells and pancreatic islet cells participate to the pathogenesis of insulin-dependent diabetes mellitus and its prevention by a suitable dietary manipulation.

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NICOTINAMIDE DOES NOT PREVENT DEVELOPMENT OF DITHIZONE-INDUCED DIABETES IN RABBITS

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It has been shown that nicotinamide (NA), an inhibitor of poly (ADP-ribose)synthase and free radical scavenger, protects pancreatic beta cells against a variety of toxic or immunemediated insults. **The aim** of the present study was to evaluate the impact of NA on the development of dithizone (D)-induced absolute insulin insufficiency in rabbits. **Materials and methods:** Male chinchilla rabbits were injected D (35 mg/kg i.v.). NA (250 mg/kg/day) was given per os 7 days prior and 7 days after D-injection. Control diabetic (CD) and intact (C) animals received vehicle alone. Blood glucose and insulin were monitored at days 3, 5, 7 after diabetes induction. Oxidative status was estimated in plasma by spectrophotometric determination of malonic dialdehyde (MDA) and diene conjugates (DC) contents. **Results:** At the end of experiment D-injected rabbits become hyperglycaemic and hypoinsulinaemic. Administration of NA did not reduce elevation in basal blood glucose levels (19.4±4.0 vs CD: 24.0±2.9, NS; C: 4.7±0.2 mmol/l) and did not affect plasma insulin contents. However, treatment with NA significantly diminished lipid peroxidation reducing MDA contents (0.53±0.07 vs CD: 1.4±0.3, $p<0.02$; C: 0.63±0.02 µmol/ml) and DC levels (0.34±0.02 vs CD: 0.41±0.01, $p<0.01$; C: 0.28±0.02 µmol/ml) compared to diabetic controls. **Conclusions:** These findings suggest that NA supplementation decreases oxidative stress, but does not prevent the development of absolute insulin deficiency in dithizone-treated rabbits.

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TROGLITAZONE INHIBITS THE DEVELOPMENT OF DIABETES AND BETA CELL ICAM-1 EXPRESSION IN NOD MICE

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Troglitazone (TGZ) is a recently developed compound for the treatment of insulin resistance. **Aims:** This study was designed to investigate whether the drug exerts anti-inflammatory properties in an animal model of type 1-diabetes. **Materials and Methods:** Female NOD mice (n=20) were treated with 500 mg TGZ kg^{-1} d^{-1} (administered with drinking water) from 4 to 35 weeks of age (TGZ-Group). Age- and sex-matched controls were either placebo-treated (P-Group, n=20) or remained untreated (U-Group, n=10) for the same period of time. Body weight and urinary glucose were monitored twice weekly. In case of diabetes manifestation (blood glucose readings ≥12 mmol/l at two consecutive occasions), the mice were treated during the remaining study interval with insulin implants. At the end of the follow-up, islets were isolated from pancreatic specimen on a single donor basis and cultured for 24 h in the presence of either interleukin 1-beta (IL-1, 20 U/ml) or TGZ (10 µM) or TGZ+IL-1, or they remained untreated (control, C). Single islet cells were analyzed by flow cytometry after immunostaining for the adhesion molecule ICAM-1. **Results:** There were no significant differences in body weight gain between the three groups. Diabetes incidence and age at manifestation were 40% and 140±18 d in the U-Group compared to 30% and 160±11 d in the P-Group. TGZ treatment significantly reduced the incidence of diabetes (5%) and delayed the onset of the disease to 182±0 d. In all three groups of animals, in vitro exposure of islets to TGZ alone did not influence ICAM-1 expression, but IL-1 treatment resulted in an up-regulation of the adhesion molecule (C- vs. IL-1-treated islets: U-Group 3.6±2.0 vs. 35.9±5.2, P-Group 3.7±0.7 vs. 38.2±2.5% positive islet cells, $p<0.01$). Islets of the U- and the P-Group responded identical to those of in vivo TGZ-treated animals (5.8±1.0 vs. 43.1±2.1% positive cells, $p<0.01$). The IL-1-induced expression of ICAM-1 (=100%) was significantly inhibited by TGZ co-culture of islets obtained from all three in vivo treatment groups. This inhibition was 34.9±3.5% in islets from untreated and 32.2±2.4% in those from placebo-treated control animals, however, corresponded to 57.8±3.1% inhibition in islets from in vivo TGZ-treated NOD mice ($p<0.01$). **Conclusions**: TGZ may suppress cell-mediated immune destruction of ß-cells in NOD mice. The observed down-regulation of adhesion molecules on target cells is a putative mechanism to reduce the binding of autoaggressive immune cells.

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This abstract has been withdrawn

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Autoimmunity in relatives of children with diabetes: a suitable case for screening?

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Aims: To determine the distribution of coeliac disease (CD) associated autoimmunity and its assocation with markers of thyroid and islet autoimmunity in patients with Type 1 diabetes and their families. **Methods:** We have investigated the prevalence of autoantibodies to the endomysial antigen human tissue transglutaminase and thyroid microsomal antibodies (MCHA) in 1442 non-diabetic first-degree relatives and 433 probands with type 1 diabetes diagnosed before the age of 21 years. IgA antibodies to ^{35}S-labelled human transglutaminase (TGA) were measured by radiobinding assay using a threshold defined by the 97.5th centile of 347 schoolchildren which achieved 96% sensitivity for untreated CD. MCHA were assayed by haemagglutination. All samples were tested for GAD, IA-2 and insulin autoantibodies by radiobinding assay and for ICA. **Results:** 63 (14.5%) of probands (median age 12.2, range 1 - 22 years), 6.5% of siblings (age 13.1, 0 - 31) ($p<0.0001$ vs probands), 8.0% of fathers and 9.8% of mothers had TGA ≥ 97.5th centile. 7.1% of probands, 6.2% of siblings, 12.9% of fathers and 23.0% of mothers had MCHA with titres ≥ 1/400. 2.5% of siblings, 1.7% of fathers and 1.1% of mothers had ≥2 islet autoantibody markers ≥ 97.5th centile. 12.4% of MCHA+ relatives vs. 7.2% of MCHA- relatives had TGA ($p=0.014$) and 7.7% of ≥2 islet antibodies+ vs. 8% of ≥2 islet antibodies- ($p=0.96$). **Conclusion:** Coeliac disease associated autoimmunity is strongly associated with overt diabetes but not associated with markers of islet autoimmunity in non-diabetic relatives. Autoimmunity to thyroid, small intestine and islet is prevalent in first degree relatives of children, but overlap between the three targets of autoimmunity is limited. Relatives are at high risk of autoimmune diseases other than type 1 diabetes and screening for these might be justified.

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MATURATION OF THE HUMORAL AUTOIMMUNE RESPONSE TO EPITOPES OF GAD IN PRE-CLINICAL CHILDHOOD TYPE 1 DIABETES.

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Aims: To examine the maturation of the humoral response to GAD epitopes. **Materials and Methods:** Epitope reactivity against GAD65, GAD67, and GAD65/67 chimeras was measured by radio-binding assay in sequential samples from birth to diabetes onset or current follow-up in 19 GAD antibody positive offspring of parents with diabetes from the BABYDIAB study. **Results:** In all offspring, the first detectable antibodies were against epitopes within GAD65 residues 96-444. In four offspring, this was the only initial reactivity. In the remaining 15 offspring, epitopes within GAD65 carboxy terminal residues 445-585 were also recognized in this initial reactivity; three had also antibodies to epitopes in GAD65 amino terminal residues 1-95, and four to GAD67, including GAD67 specific epitopes. Subsequent spreading of epitope reactivity was seen in 9 of 12 offsprings with follow-up samples. Spreading was mostly (7 cases) to amino terminal GAD65 epitopes. None of the epitope reactivities, nor changes in reactivity correlated with diabetes onset in the 9 offspring who have developed disease. **Conclusions:** The findings show that the humoral autoimmune response to GAD found in childhood is dynamic, is initially against epitopes within the middle portion of GAD65, suggesting that this region is a primary target of autoimmunity, and spreads to epitopes in other regions of GAD65 and GAD67.

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HIGH PREVALENCE OF GAD-A AND IA2-A IN THE SERA OF PATIENTS WITH LAMBERT EATON MYASTHENIC SYNDROME AND/OR SMALL CELL LUNG CARCINOMA

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Aims: Despite endodermal origin β cells share numerous features with neural tissues and some immunoreactivity may occur in autoimmune neurological diseases, as in Stiff Man Syndrome. **Material and methods:** We looked for the presence of diabetes-specific autoantibodies GAD-A and IA2-A in neurological syndromes with authenticated or supposed autoimmune origin: Lambert Eaton Myasthenic Syndrome (LEMS), n=30, Lateral Amyotrophic Sclerosis (LAS), n=17, Multiple sclerosis (MS), n=30 with a sensitive and specific RIA. **Results:** There was a high frequency of these Ab in LEMS (GAD-A: 35%, IA2-A: 21%, double+: 18%) in comparison to other neurological diseases; LAS: 18, 12 and 12%, MS: 10, 3 and 3% respectively. In newly diagnosed LEMS, GAD-A were more frequent in paraneoplastic aetiology (GAD-A: 5/8 vs. 1/8 in idiopathic syndrome, $p<0.05$), and IA2-A (2/8 vs. 0/8 respectively). The difference was not observed in long-standing LEMS (GAD-A: paraneoplastic 5 /11, idiopathic 3/7). Among LEMS patients only one was treated for IDDM. Cross-reactivity between diabetes-specific Ab and Ca^{2+} channel Ab (types N and P/Q) was excluded as there was no association between the presence or the titre of these Ab in LEMS and in the contrary anti Ca^{2+} channel (type N) Ab were found in only one serum among 34 GAD-A+ sera from IDDM patients. Finally, these Ab were observed in some sera of patients bearing a small cell lung carcinoma (SCLC) without LEMS as double positivity was noted in 2 sera among 9. **Conclusion:** LEMS, as Stiff Man Syndrome, is a neurological disease characterised by a high incidence of GAD-A and IA2-A.These humoral markers can be used with Ca^{2+} channel Ab in the diagnosis of this syndrome. Expression of GAD and IA2 or related epitopes by SCLC could be responsible for this immunoreactivity

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EARLY AUTOANTIBODY RESPONSES IN PRE-TYPE 1 DIABETES ARE IgG1 DOMINATED AND SUGGEST DETERMINANT SPECIFIC REGULATION

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Aims: ß-cell destruction in animal models is mediated through a T-helper 1 dominated autoimmune response and measurements of antigen specific antibody subclasses have been used to identify periods of Th1- vs Th2-dominant immunity. The aim of this study was to characterize the humoral islet autoimmunity of early type 1 diabetes in man. **Methods:** IgG subclass antibodies were measured sequentially from birth to diabetes onset or current follow-up in 26 autoantibody positive offspring of parents with type 1 diabetes using the protein A/G RBA of IAA, GADA, and IA2A with IgG subclass-specific sepharose beads for antibody detection. **Results:** Islet autoantibody appearance was characterized by early peak titres of IgG1 antibodies to one or more antigens at a median age of 2.2 yrs (inter-quartile range 2-2.9 yrs). IgG1-IAA peaks preceded other islet antibodies in 13 offspring, 10 having HLA DR4, while a peak response to GAD was found prior to IAA in 5 cases, 4 of whom had HLA DR3. In 5 offspring, an acute diabetes onset occurred during the initial peak antibody response. In the remainder, early IgG1 antibody levels declined markedly and antibody peaks against other ß-cell antigens arose sequentially over several years. Second peak antibody responses to the same antigen were seen in only two offspring, both developing diabetes at this time. Two others developed diabetes with declining antibody titres. Other IgG subclass responses were usually of much lower titre. The IgG4 response to insulin was exceptional, being detected in 13 offspring, dominant over IgG1 in 4 of these and in 5 appeared and/or persisted after IgG1 levels declined. IgG4 responses were not associated with diabetes protection. **Conclusions:** The data suggest that type 1 diabetes has an early acute destructive phase of ß-cell autoimmunity, which can be regulated and which spreads chronically until diabetes onset.

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PREVALENCE OF GADA65 IN SIBLINGS OF SARDINIAN TYPE 2 FAMILIES

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On behalf of The Study Group for the Genetics of Diabetes in Sardinia (SGGDS)

Background Adults type 1 can masquerade as type 2 at presentation, with a slow deterioration in metabolic control and later progress to insulin dependency : LADA. In Sardinia where the incidence of type 1 is very high in children it's possible that LADA might be a significant proportion of diabetes in adults. **Objective** In the present study we investigate the prevalence of glutamic acid decarboxilase antibodies (GADA65), in a group of Sardinian siblings affected or not by type 2 diabetes mellitus. **Patients and methods** Diabetic (D) and non-diabetic (ND) siblings from families with at least two affected were enrolled . The non diabetic spouses of the siblings willing to participate as "control" (C) were also enrolled. Type 2 diabetic subjects (known age at onset of diabetes between 30 and 69 years) were enrolled following the WHO criteria, additionally in insulin treated patients, insulin was not used during the first three years after diagnosis. **Results** We present the results in subjects with at least 4 years of known diabetes as compared to non diabetic siblings and controls. Data are mean ± esm. No significant difference for sex, BMI and age at diagnosis were present between diabetic subjects positive (P) and negative (N) for GAD65. *p=0.03 vs type 2 on diet or on Oral Hypoglicemic Agents (OHA)

	DIABETES duration	P/N(%)	$\chi 2$	P vs controls
C (n=204)	----------	5/199(2.5)	----------	----------
ND (n=80)	----------	4/76(5.0)	0.52	0.467
D diet (n=228)	11.4±1..4	12/216(5.3	1.57	0.212
D OHA (n=239)	11.4±1.01	11/228(4.6)	0.91	0.339
D OHA+Insulin (58)	15.9±3.4 *	2/56(3.4)	0.02	0.962
D insulin (n=79)	16.0±1.2 *	13/66(16.5)	16.5	<0.0001

Conclusion Our data are consistent with the hypothesis that in Sardinia a substantial proportion of insulin treated type 2 diabetic patients (in whom insulin has been started after more than three years from the diagnosis) are positive for GAD65, indicating those as LADA. These data must be taken into consideration in studies aiming at the discovery of the etiology of type 2 diabetes.

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CROSS SECTIONAL AND LONGITUDINAL STUDY FOR THE NON-OBESE DIABETIC PATIENTS WITH GAD ANTIBODY

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Aims: The characteristic feature of diabetes mellitus(DM) in Korea is that 70-80% of diabetic patients are non-obese, adult-onset type and type 1 is very rare(below 1%). Autoantibodies to glutamic acid decarboxylase (GAD) can occur in apparently typical, non-insulin dependent diabetes mellitus (type 2). However, the role of autoantibody to GAD in type 2 DM is not known. We determined the characterization between GAD positive and negative non-obese, adult-onset diabetes in Korea and followed up the patients. **Materials and Methods:** We measured autoantibdy to GAD in 662 patients and followed between 1-2 years later. **Results:** The body mass index(BMI) of the patients were below 24 and onset age were between 30 and 77 years old. Compared to the antibody-negative group (n=595; 89.9%), patients with antibody-positive (n=67; 10.1%) were significantly lower c-peptide levels (fasting c-peptide levels: 0.98±0.68 vs. 1.23±0.67 ng/ml, $p<0.05$, postprandial c-peptide levels: 1.41±0.91 vs. 2.13±1.71 ng/ml, $p<0.05$) and significantly younger (median age: 44.1±12.7 vs. 50.8±12.3 years, $p<0.05$). Fasting and postprandial c-peptide were significantly decreased 1-2 years later($P<0.05$). GAD-positive group was a lower BMI (21.4±3.3 vs. 23.5±3.7, $p<0.05$) and more frequent insulin requiring (72% vs. 31%, $p<0.05$). GAD positive patients had higher frequencies of weight loss (56% vs. 21%, $p<0.05$) and ketonuria (45% vs. 14%, $p<0.05$), but lower prevalence of hypertension (32% vs. 47%, $p<0.05$). There were no significant differencs in sex, lipid profile and family history of diabetes. **Conclusions:** The presence of autoantibody to GAD allows us to identify the group with more deteriorated β-cell function and more frequent insulin-requiring, but lower prevalence of obesity, dyslipidemia and hypertention.

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DIFFERENT ONSETS OF TYPE 1 DIABETES. AGED-BASED IMMUNOLOGICAL AND CLINICAL DIFFERENCES.

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AIMS: To study, within our environment, the prevalence of autoantibody markers (ICA, GAD, IA-2) at diagnosis of type 1 diabetes (DM 1), comparing children and adults. To classify patients on the basis of the presence/absence of the mentioned markers, and to confirm possible clinical differences. **MATERIALS AND METHODS**: ICA (by indirect immunofluorescence) and GAD, IA-2 (RIA) were studied in 68 newly diagnosed DM 1 patients (42 male/ 26 female); it was considered idiopatic when all studied antibodies were negative (at least two determined). The study group of 25 children (mean age 6.4 ± 3.9) was compared with the study group of 42 adults (mean age 23 ± 5.2). The study was completed with the comparison of the clinical and analytical characteristics. The statistical analysis was performed with t-student and chi square. **RESULTS**: 36 adults and 21 children were diagnosed autoimmune DM 1 (85.7%). The prevalence of GAD + was significantly higher in adult patients (75.6 % vs. 52 %, p<0.001). The prevalence of IA-2 + was significantly higher in the children study group (65% vs 40%, p<0.001) compared with the study group of adults. The ICA positivity did not change between both groups. IA-2 and ICA were more positive in female patients, whereas in GAD there was no difference related to sex. **CONCLUSIONS**: IA-2 is considered as the best marker for DM 1 in children, whereas GAD had a highest sensitivity in adults. Autoimmune DM 1 is found in 85.7 % of adult patients and 80.8 % of children recently diagnosed with DM type 1. The determination of GAD and IA-2 shows the highest percentage of positivity (96 %).

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PREDICTIVE VALUE OF GAD_{65}Ab AND IA-2Ab IN TWO GROUPS OF TYPE 1 DIABETES MELLITUS FOR ASSOCIATED AUTOIMMUNITY

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Aim: To determine the correlation between islet-related, thyroid and α-gliadin autoantibodies in two groups of Type 1 diabetes mellitus (DM-1) patients. **Patients:** Group 1 (juvenile type, n=25, age 31.8±5.4 y, 11/F/14M, duration of DM 14±10.4 y) and group 2 (LADA type, n=22, 52.5±8.6 y, 13F/9M, 8.6±9.6 y resp.). **Methods:** Serum C-peptide: RIA (Immunotech), IA-2Ab: IRMA (Brahms Diagnostica), GAD_{65}Ab: ELISA (Roche Boehringer M., upper normal limit 30 ng/ml), anti-α-gliadin IgA and IgG: ELISA based on our own antigen preparation and Tg-Ab and TPO-Ab autoantibodies: ELISA (Alpha Dialab). **Results:** Groups 1 and 2 differed in basal and stimulated C-peptide levels (0.13±0.11 vs. 0.55±0.3 resp. 0.21±0.11 vs. 0.88±0.43 nmol/l, p<0.01). Group 1: We found GAD_{65}Ab values above 30 ng/ml in 76% (36.9% over 1000 ng/ml) and IA-2Ab positivity in 33.4%. Only 37% GAD_{65}Ab positive patients were also IA-2Ab positive. 85.7% patients with elevation of the both B-cells autoantibodies revealed increased levels of Tg-Ab and/or TPO-Ab and 71.4% IgA and/or IgG α-gliadin autoantibodies. Group 2: GAD_{65}Ab positivity in 86.4% (36.4% over 1000 ng/ml), IA-2Ab positivity in 36.4%. Increased levels of the both autoantibodies were detected in 77.8% patients, but only 57.1% of them were Tg-Ab and/or TPO-Ab positive and 22.7% have increased levels of IgA/IgG α-gliadin-Ab. However, 7/22 persons in group 2 vs. 3/25 in group 1 had the highest levels of the all antibodies and all these patients suffered from manifest thyroid disease with altered thyroid function. The possible clinical importance of associated α-gliadin IgA/IgG antibodies is discussed. **Conclusions: The predictive value of GAD_{65}Ab is in contrast to IA-2Ab much higher regarding especially thyroid autoimmunity. In this study no significant correlation between these islet autoantibodies was found. We recommend to examinate often associated thyroid and gastrointestinal autoimmune diseases especially in GAD_{65}Ab+IA-2Ab positive diabetic patients, mainly with the LADA type.**

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GAD ANTIBODIES IN AUSTRIAN TYPE-2 DIABETIC PATIENTS

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The prevalence of GAD antibodies seems to be relatively high in type-2 diabetic patients of northern European countries, but relatively low in South Europeans.

Aims: Since no information is available about Austrian type-2 diabetic patients GAD antibodies (GADA) were studied in 390 patients whose diabetes was diagnosed at a mean age of 52 years. At the time of evaluation 204 patients were treated with insulin and 186 were on oral hypoglycemic agents.

Results: High levels of GADA (>30 U/ml) were found in 28 (7,1%) and low levels (1,5-30 U/ml) in 33 (8,3%) of 390 type-2 diabetic patients. GADA were found in 43 (21.1%) out of 204 insulin treated patients, but in only 18 (9.7%) out of the 186 patients without insulin treatment. GADA positivity (n=43) in the insulin treated patients was associated with a low BMI (p<0.001) and short diabetes duration before insulin treatment (p<0.001). In patients without insulin treatment clinical characteristics of the patients were very similar in GADA positive and GADA negative patients.

	with Insulin treatment			without Insulin treatment		
	GADA neg	GADA pos		GADA neg	GADA pos	
Number	161	43	p-value	168	18	p-value
GADA (U/ml)	0.5±0.3	41±32	0.0001	0.5±0.3	29±31	0.0001
Age at onset(yrs)	52±12	54±12	n.s.	52±12	51±11	n.s.
BMI (kg/m^2)	28±5	24±4	0.0001	30±5	30±5	n.s.
Duration of diabetes (yrs)	14±9	11±9	0.05	6±6	8±8	n.s.
Years without insulin	11±8	5±6	0.0001	-	-	

Conclusion: Prospective long term studies are needed for clarification, why some GADA positive patients progress to insulin deficiency, whereas other patients with high GADA can be treated sufficiently for many years with oral hypoglycemic agents.

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IVGTT AND ANTI-GAD65 ANTIBODIES IN THE DIAGNOSIS OF GLUCOSE INTOLERANCE

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Aim: The aim of the study was to evaluate the impact of first and second phases of insulin secrtion, assessed during IVGTT, and anti-GAD65 antibodies as β-cell autoimmune marker, in the early diagnosis of the type of glucose intolerance in patients aged 30-45 years. Materials and Methods: 54 patients (32 males and 22 females) were enrolled in the study. They were divided into two groups according to OGTT: diabetic patients (n=30, mean age 39.1±8.9years) and subjects with IGT (n=24, mean age 39.0±10.1years). A group of healthy volunteers (n=19, mean age 37.9±7.4years) served as a control group. First phase insulin secretion (FPIS) was defined as the sum of insulin levels at 1st and 3rd minute (values > 48mIU/l were considered normal) and second phase (SPIS) - by the values at 30th and 60th minute during IVGTT. Results: Using the data from IVGTT and anti-GAD65 antibodies, the diabetic patients were divided into two groups: type 1 diabetic patients (n=12, showing loss of FPIS and SPIS - 9.53±3.8mIU/l and 10.6±2.4mIU/l, respectively, as well as high prevalence of anti-GAD65 antibodies - 81%) and type 2 diabetic patients (n=18, who demonstrated declined FPIS - 25.34±3.8mIU/l, p<0.001 as compared to the healthy controls and p<0.02 as compared to type 1 diabetic patients, and relatively preserved but retarded SPIS - 68.8±11.7mIU/l, none of them being anti-GAD65 positive). As far as their response during IVGTT is concerned, the subjects with IGT were divided into two subgroups: with normal FPIS (n=15, 119.4±29.8mIU/l), and with reduced FPIS (n=9, 29.2±6.4mIU/l, p<0.001 as compared to the healthy controls and p<0.01 as compared to type 1 diabetic patients), a significant percentage of the two subgroups (47% and 88%, respectively) being anti-GAD65 positive. Conclusions: IVGTT allows precise assessment of the phases of insulin secretion and, in combination with anti-GAD65 antibodies, helps to set proper early diagnosis of the type of diabetes in newly diagnosed patients aged 30-45 years. This approach appears to be of great clinical importance in the adjustment of prompt adequate treatment.

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ISLET CELL AND THYRO-GASTRIC AUTOIMMUNITY IN TYPE 1 DIABETES: ASSOCIATION BETWEEN GADA AND ICA, IAA AND PCA.

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Type 1 diabetes mellitus results from autoimmune destruction of insulin-producing β-cells. These patients also present with elevated levels of auto-antibodies to the thyroid gland (TPO) and to the gastric mucosa (PCA). AIMS: To study the prevalence of and the relationship between β-cell and thyro-gastric autoimmunity in type 1 diabetes. PATIENTS AND METHODS: A total of 171 type 1 diabetic patients (male/female: 86/85) were studied (mean age: 19.3 ± 10.6 y, duration of diabetes: 4.8 ± 3.7 y, HbA1c: 8.0 ± 1.5%). Autoimmune aggression to the β-cell was measured with ICA (islet cell antibodies, pos ≥12 JDFU), IAA (insulin antibodies, pos ≥0.7 %) and GADA (glutamic acid decarboxylase Ab, pos >0.009). Anti-thyroid peroxidase (TPO, pos >100 U/ml) and gastric parietal cell antibodies (PCA, pos >1/20 dilution) were also assayed. RESULTS: The majority of subjects (74.3%) showed 2 or more of the above-mentioned auto-antibodies (AAB) and only 11 patients had none. The prevalence rates were: IAA: 81.3%, GADA: 64.9%, ICA: 46.2%, PCA: 19.9% and TPO: 19.3%. In patients with ICA persisting more than 3y, the prevalence of IAA+ (p=0.01, OR: 4.33) and GADA+ (p=0.03, OR: 2.40) was higher than in subjects with ICA persisting less than 3y. The titer of ICA correlated with the number of AAB (p=0.001, r=0.25) and the duration of diabetes (p=0.03,r= -0.17). GADA positivity was associated with ICA+ (p=0.006, OR: 2.54) and IA+ (p=0.007, OR: 2.97). We also observed a higher prevalence of GADA in PCA+ patients compared to PCA- subjects (p=0.005, OR: 3.89). Age at onset of diabetes was higher in PCA+ subjects (p=0.04) and they were older (p=0.04) than PCA- diabetics. The presence of TPO did not correlate with any other AAB, although a trend of association was observed with persisting ICA+. CONCLUSIONS: Particularly in patients with persisting ICA, screening for thyro-gastric AAB is useful for early detection of polyendocrine autoimmunity. GADA positivity is associated with ICA and IAA. In addition, the presence of GADA and an older age at diagnosis of diabetes may be helpful in predicting PCA positivity.

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HIGH PREVALENCE OF THYROID-ANTIBODIES IN FIRST-DEGREE RELATIVES OF IDDM PATIENS WITH ISLET-AUTOANTIBODY POSITIVITY.

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Thyroid autoimmunity is often associated with type I diabetes mellitus. We wished to study the prevalence of thyroid antibodies (TG/TPO) in first-degree relatives of IDDM patients and examine their correlation with islet autoantibody positivity. We determined ICA, IA-2, GAD65 and thyroid-Abs in 1436 first-degree relatives (mean age 26.4±16.2 yrs) of IDDM patients. The distribution of the relatives was: parents 594, (group A) and siblings/children 842, (group B). Thyroid-Abs were found positive in 55 cases overall, 27 (4.5%) in group A and 28 (3.3%) in group B. Multivariate method of discriminant analysis, revealed that high levels of GAD65 / IA-2 Abs could predict the thyroid antibody positivity for the group B. The predictive value was from 92.9% to 94.7% and a significant correlation was observed between GAD65 / IA-2 Ab levels and thyroid-Ab positivity (r=0.65, p<0.001) in this group. In contrast, in group A the predictive value of GAD65 / IA-2 antibody levels to thyroid antibody positivity was much lower, 29.3% - 40.7%. The presence of GAD65 / IA-2 Abs is predicting of thyroid-Ab positivity in first-degree relatives, siblings and children, of IDDM patients. Thus, screening for thyroid-Abs is recommended in this population.

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ANTIBODY RESPONSE TO PROTEIN PEPTIDES AND POLYSACCHARIDE ANTIGENS IN PATIENTS WITH TYPE-1 AND TYPE-2 DIABETES

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Epidemiological studies have shown that patients with diabetes mellitus have an increased risk for infectious diseases. This could be explained by an impairment in immune regulation. The immune response can be estimated by the antibody response after vaccination. However, data addressing AB response following immunization are conflicting in patients with diabetes.
Aims: In the present ongoing study the immune response following vaccination with different types of antigens was investigated in patients with type-1 and type-2 diabetes.
Method: Antibodies were determined by ELISA. An interim analysis of 17 patients with type-1 diabetes and 16 age-matched controls (age:32±8 vs 30 ± 4 yrs) as well as 18 patients with type-2 diabetes compared to 14 controls (age: 56 ± 8 vs 52 ± 10 yrs) has been performed.
Results: Patients with type-1 diabetes showed significantly decreased diphtheria-antibody levels after a single immunization with diphtheria toxoid vaccine in patients with type-1 diabetes compared to controls: (Diphteria antibodies, IU/ml:1,8±1,9 vs. 5,1 ± 4,9; p<0,01) as well as after immunization with hepatitis A vaccine in seronegative individuals (HAV-Antibodies, IU/ml: patients 92,6 ± 119, vs. 271 ± 292; p< 0,009). In contrast in patients with type-2 diabetes immune response to diphteria-toxoid was comparable to controls. Response to polysaccharide antigens (haemophilus influenzae, streptococcus pneumoniae) was comparable to controls in both patients groups.
Conclusion: These findings indicate that patients with type-1 diabetes have an impaired capacity to produce IgG antibodies following vaccination with clinically relevant protein antigens, whereas response to polysaccharide antigen is unaltered. This may implicate that vaccination schedules should be adapted for this patient group in order to achieve sufficient protection.

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ANTI-P53 AUTOANTIBODIES IN PATIENTS WITH IDDM

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Aims: Since autoantibodies against the p53 tumor suppressor protein, which is also implicated in the apoptosis signaling, have been described in sera from patients with autoimmune diseases (LES, Sjögren's syndrome, systemic sclerosis); we investigated the presence of autoantibodies to p53 protein in patients with IDDM. **Methods:** Antibodies specific for p53 protein (anti-p53Ab) were measured by an ELISA procedure using recombinant wild-type human p53 in sera from 47 newly diagnosed IDDM patients (mean disease duration 0.3 months) subdivided into two groups according to the age: 25 aged < 14 years (10M/15F; mean age 8 years, range 1-14 years) and 22 aged > 14 years (12M/10F; mean age 27.2 years, range 18-39 years); and from 20 age and sex-matched control subjects. **Results:** Anti-p53Ab were detected in 4/25 (16%) patients aged < 14 years, in 2/22 (9,1%) patients aged > 14 years and in 0/20 healthy controls respectively. All 6 anti-p53Ab positive sera were ICA+; 4 were IA2Ab+, 1 GAD65Ab+ and the other one IA2Ab-/GAD65Ab-. Anti-p53 autoantibodies persisted only in one of 4 patients whose subsequent serum sample was available (range of sampling period 2-36 months), while ICA persisted in all. **Conclusion:** These first data on anti-p53Ab in some newly diagnosed IDDM patients may reflect the involvement of p53 gene products in the modulation of apoptosis during islet infiltration and beta cell destruction leading to IDDM.

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INSULIN ANTIBODY RESPONSES WITH INSULIN LISPRO IN LONG TERM STUDIES IN PATIENTS WITH TYPE 1 OR TYPE 2 DIABETES
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Aims: To evaluate the long-term insulin antibody response to insulin lispro (Humalog®) in patients with either type 1 or type 2 diabetes. **Materials and Methods:** 771 patients from 105 investigators in 19 countries participated in a non-controlled, 4 year extension study following randomized, controlled insulin lispro clinical trials. All patients received insulin lispro before each meal and a basal insulin appropriate for the patient's metabolic needs. Measurements were obtained for lispro-specific, human insulin-specific and cross-reactive antibodies (CRA) at 3 month intervals for the first year and yearly thereafter. The antibodies were measured at two central laboratories and the data were combined by using a cumulative distribution function transformation. Appropriate reference ranges for the antibody measurements were estimated using antibody measurements from patients previously exposed to insulin. **Results:** 85% (653/771) of the patients completed this long term study and received insulin lispro from 3 to 4 years. The antibody response for lispro-specific and human insulin-specific antibodies was low with only a few patients achieving levels outside of the reference ranges. For the CRA measurements, the median values at baseline were 3.9% for type 1 and 2.1% for type 2 patients. After 3 years of insulin lispro therapy the median CRA values were 4.1% for type 1 and 2.4% for type 2. The median difference from baseline for CRA for type 1 patients was 0.54% (interquartile range of -0.9% to 2.9%) and for type 2 patients was –0.40% (interquartile range of –3.0% to 0.7%). Only a few patients had a significant CRA response at their last visit during the study (1.4% for type 1 patients and 0.8% for type 2 patients) when defined as a value greater than four times the baseline measurement and above the upper reference range. **Conclusions:** Specific and cross reactive antibody measurements conducted in long term clinical trials demonstrate that therapy with insulin lispro does not result in an enhanced immunogenic response.

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INDUCTION OF ISLET-CELL SPECIFIC AUTOANTIBODIES UNDER IMMUNSUPPRESSIVE THERAPY WITH TACROLIMUS/FK506
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Aims: Tacrolimus/FK506 is a widely used immunsuppressive drug in rejection therapy after organ transplantation. The induction of diabetes mellitus is a known side effect of this therapy but the mechanism for it remains obscure. This study investigated whether induction of islet-cell specific autoimmunity may contribute to diabetes induction by FK506.
Materials and Methods: 22 patients treated with FK506 and 20 patients treated with cyclosporine A (CsA) after liver transplantation were tested for islet cell antibodies (ICA) by indirect immunofluorescence and for antibodies against glutamic acid decarboxylase 65 (GAD65) and tyrosine phosphatase IA2 by radioimmunoassays. All antibody assays were validated by Islet Antibody Workshops for sensitivity, specificity and consistency.
Results: Five/22 FK506-treated compared to 0/20 CsA-treated patients had ICA (5-320 JDF-U; $p<0.03$). Of the 5 ICA-positive patients, 3 had diabetes (2 newly developed diabetes after transplantation) and 2 normal oGTT. At least one ICA-positive patient was definitely ICA-negative before transplantation. Three of the 5 ICA-positive patients compared to 2/17 ICA-negative patients treated with FK506 had the diabetes associated HLA haplotype DQB1*0302 ($p<0.01$). One patient of each group had GAD- and IA2-Ab.
Conclusions: Immunsuppressive therapy with FK506 was associated with a higher frequency of islet cell specific autoimmunity measured by ICA compared to conventional CsA therapy. At least in one case ICA seem to be triggered de-novo after transplantation despite FK506 therapy. ICA were associated with diabetes associated HLA haplotypes. These results raise a further concern for usage of FK506 as first line immunsuppressive therapy in diabetes prone individuals e.g. after pancreas transplantation.

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A TIME-RESOLVED FLUOROIMMUNOASSAY FOR HUMAN SERUM IMMUNOREACTIVE INSULIN BASED ON TWO MONOCLONAL ANTIBODIES
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Aims To develop an accurate, sensitive and rapid time-resolved fluoroimmunoassay (TRFIA) for human serum immunoreactive insulin **Materials and Methods** The assay was a 'sandwich type' immunoassay using two monoclonal antibodies (MoAbs) and was performed with the DELFIA system (Wallac) Anti-human insulin MoAbs were prepared by immunizing Balb/c mice with semi-biosynthetic human insulin (Novo) Two MoAbs recognizing different epitopes of insulin were screened for the assay One of the antibodies was bound to the bottom of 96-well microtiter plates and the other was labeled by europium (Eu^3-) **Results** Serum samples of 50μl could be analyzed within a concentration range of 6 25μIU/ml to 100μIU/ml The detection limit of this assay, 2.1μIU/ml, was comparable to the conventional RIA and results overall correlate well with those of the PAMIA (Sysmex, RIN-700A, Japan) (r^2=0 965). **Conclusions** The insulin TRFIA has several advantages over the standard insulin RIA These include (1) avoidance of hazards and inconvenience of handling radioactivity, (2) not requiring a separate test tube for each sample, (3) stability of the Eu^3-labeled MoAb (>6 months), (4) short time period required for the assay (<4 hours), and (5) the possibility of long-term storage (at least 3 months) of antibody-coated microtiter plates

Key words TRFIA Human insulin Monoclonal antibody

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AUTOIMMUNITY TO TISSUE TRANGLUTAMINASE C IN PATIENTS WITH TYPE 1 DIABETES
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Aims: silent Coeliac disease is a gluten driven autoimmune disease which is relatively frequent in patients with type 1 diabetes. The aim of this study was to determine the extent of gluten associated autoimmunity in type 1 diabetes **Material and methods**: IgG and IgA autoantibodies against human transglutaminase C (TGCA) using novel radiobainding assays were measured in a cohort of 290 patients with new onset type 1 diabetes and 213 control subjects. **Results**: 24 (8%) patients with type 1 diabetes had both IgA and IgG TGCA, 1 had only IgA TGCA, and 97 (33%) had IgG TGCA only. Antibody levels in those with IgG TGCA only were relatively low. Only 2 (1%) control subjects had TGCA. IgA TGCA were associated with the HLA DQA1*0501 DQB1*0201 heterodimer, while IgG TGCA were associated with HLA DRB1*04. Of 11 patients with TGCA who have had intestinal biopsies, 9 showed a flat mucosa typical of Coeliac disease. In another 5 patients without TGCA at diabetes onset, TGCA and a flat mucosa was found on follow-up 1-3 years later. **Conclusion**: These data show that almost 10% of patients have autoimmunity typical of Coeliac disease and another 30% have low level gluten associated IgG TGCA autoimmunity. This high prevalence suggests either a direct involvement of the gut in the pathogenesis of type 1 diabetes, or that transglutaminase is a secondary autoantigen resulting from beta cell destruction.

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ANTIBODIES TO GLIADIN AND ENDOMYSIUM IN TYPE 1 DIABETES.
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The prevalence of coeliac disease (CD) in adult population is unknown because silent and latent stages do exist. Type 1 diabetes mellitus (IDDM) may be associated with CD because of common genetic background and/or shared pathogenetic mechanisms. We investigated 74 probands with IDDM (32±11 y, disease duration 13±9 y), 69 parents of diabetic probands (56±10 y), 59 siblings (30±11 y) and 50 healthy controls (35±10 y) for the presence of circulating islet cell antibodies (ICA), anti-glutamic acid decarboxylase antibodies (GADA65), anti-gliadin Immunoglobulins A and G (IgA- and IgG-AGA). All patients with raised AGA, performed also IgA anti-endomysium antibody indirect immunofluorescence assay (EmA).
Samples were positive for ICA in 19 diabetics (26%), 4 parents (6%), 4 siblings (7%), 0 controls ($p<0.001$); for GADA in 34 diabetics (46%), 4 parents (6%), 1 sibling (2%), 0 controls ($p<0.001$). Had raised IgA-AGA (>4.4 mg/L) 25 IDDM patients (34%), 10 parents (14%), 5 siblings (8%), 3 controls (6%) ($p<0.001$). Had raised IgG-AGA (>18 mg/L) 4 IDDM patients (5%), 5 parents (7%), 0 siblings (0%), 4 controls (8%). Both IgA- and IgG-AGA were detected in 1 IDDM and 2 parents. The prevalence of IgA-AGA positivity in IDDM was significantly higher than in controls ($p<0.001$). Finally, 50 AGA positive subjects performed EmA test: only 2 of them resulted EmA positive, an IDDM patients and a sibling. The patients with IDDM had a small-bowel biopsy specimen consistent with CD and, as sole evidence of malabsorption, sideropenic anemia. EmA positive sibling also showed severe iron deficiency, yet refused endoscopy. We conclude that: 1) CD cannot be diagnosed on the basis of associated IgA- and IgG-AGA alone. Nevertheless, detection of such antibodies is useful, in combination with EmA, in screening for endoscopic biopsy; 2) too high rate of detection of IgA-AGA in IDDM patients in comparison with other groups excludes a false positivity of the test itself, while suggests a pathogenetic association of both immunological disorders, perhaps related to abnormal gamma/delta TCR-bearing intraepithelial lymphocytes.

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PREDOMINANCE OF THE TCR Vβ7 GENE FAMILY IN PANCREATIC AND IN CIRCULATING T-CELLS FROM TYPE I DIABETES PATIENTS
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Aims: In a previous study, high frequency of T-cell receptor (TCR) Vβ7+ T-cells was detected in lymphocytes isolated from pancreatic islets of children at the onset of Type I diabetes. In this study, we assessed whether a preferential expression of certain TCR Vβ gene families was present in peripheral blood mononuclear cells (PBMC) from patients at different time-points during Type I diabetes progression. TCR repertoire analysis was also performed on pancreatic specimens from a child who died at the onset of Type I diabetes. **Material and Methods**: A semi-quantitative PCR was used to analyze TCR usage in PBMC of 16 newly-diagnosed diabetic patients and in PBMC collected at serial time points, before, at, and after the onset of the disease on 4 first-degree relatives of Type I diabetes probands, who eventually developed diabetes while under our clinical monitoring (i.e., converters) and on 4 high-risk relatives who have not yet developed diabetes. 12 patients after 1-14 years from the onset of diabetes were also studied. HLA and age-matched controls were used. **Results:** The analysis of the TCR usage in the T-cells infiltrating the pancreas of a child at the onset of diabetes, demonstrates that the Vβ7 gene family was expressed by almost half of the lymphocytes. Same analysis in the patients' PBMC showed that 9/16 newly-diagnosed diabetic patients, 3 high risk individuals and the converters at all time points had values of Vβ7 above the upper limit of the control population ($p<0.001$, <0.05 and <0.0005, respectively). In the converters, another Vβ gene family, Vβ13.1, showed values above the upper limit of the control population. Long-standing diabetics exhibited Vβ7 and Vβ13.1 values that were not statistically significant from those detected in the control populations. **Conclusions:** Selective expansion of T-cells carrying the TCR Vβ7 gene family was observed in the pancreas of a child at the onset of diabetes and was also evident in PBMC from individuals progressing to diabetes and in newly-diagnosed patients.

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ENTEROVIRAL INFECTIONS IN UTERO AND DURING EARLY CHILDHOOD AND THEIR INFLUENCE ON THE DEVELOPMENT OF ß-CELL AUTOIMMUNITY IN OFFSPRING OF PARENTS WITH TYPE 1 DIABETES
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Aims: Previous studies reported an increased frequency of antibodies against enteroviral (EV) antigens in patients with type 1 diabetes suggesting a role for EV infections in the pathogenesis of the disease. However, it is still unknown whether EV infections are causally related to the development of ß-cell autoimmunity or merely represent scondary events in subjects already affected by autoimmunity. We therefore have prospectively evaluated EV infections prior to and in parallel with the appearance of ß-cell autoimmunity in offspring of parents with type 1 diabetes. **Methods:** Using ELISAs, IgG-antibodies (abs) against a panel of enteroviruses, and, if present, more specifically, IgG-abs to Coxsackie virus serotypes (CV) B3, CVB4 and CVB5 were measured 1) at delivery in 37 mothers of children who developed ß-cell-abs (IAA, GADA, IA-2A) later in life and in 125 mothers of children who remained ß-cell-abs negative; 2) from birth until 5 years of age in 37 offspring who developed ß-cell-abs and 46 offspring who remained ß-cell-abs negative. **Results:** a) 17% of mothers from ß-cell-abs positive vs. 18% of mothers from ß-cell-abs negative offspring had elevated EV-abs, including 8% and 8% with CVB3-, 0% and 2.4% with CVB4-, and 0% and 1.6.% with CVB5-IgG-abs, respectively. c) In offspring, 5 (13%) with and 5 (11%) without ß-cell-abs developed elevated EV-abs during follow-up. Importantly, only one child developed EV-abs, including CVB3-IgG, -B4-IgG and -B5-IgG prior to ß-cell-abs, while in all other offspring EV-abs were found in parallel or after the development of ß-cell autoimmunity. **Conclusions:** EV infections were generally a rare event and did not show a consistent temporal relationship to islet-cell autoimmunity. Our data, therefore, do not support the view that EV infections play a role in the induction of ß-cell autoimmunity in first degree relatives of patients with type 1 diabetes.

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γδ T CELLS ALTERATIONS IN THE PERIPHERAL BLOOD OF HIGH RISK DIABETES TYPE 1 SUBJECTS WITH SUBCLINICAL PANCREATIC B-CELLS IMPAIRMENT.

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There is increasing evidence that CD3+ cells bearing γδ T cell receptor (represent the minor subpopulation of the T cells in the peripheral blood in humans) are involved in autoimmunity development. γδ TCR+/CD8+ T cells have been recently found to play a critical role in the pathogenesis and prevention of autoimmune diabetes in the animal model. **Aims:** The aim of the present study was the estimation the γδ T cell subpopulation levels in the peripheral blood of subjects with preclinical and overt type 1 diabetes and their possible associations with the humoral immunity, metabolic parameters and pancreatic B-cells function. **Materials and methods:** The study was carried out in 3 groups of subjects: 26 first degree relatives of type 1 diabetes patients (prediabetics) with the combinations of autoantibodies against pancreatic B cells (ICA,GADA, IA-2A, IAA), 22 patients with a recent onset of type 1 diabetes and age and sex-matched 24 healthy volunteers (control group). The percentages of lymphocytes subsets: CD3+/CD8+, γδTCR+/CD8+, γδTCR+/CD8- were measured on flow cytometer. **Results:** We observed a decrease in the absolute numbers (and percentages) of γδ +/CD8+ and γδ+/CD8- T cell subpopulations in peripheral blood in the prediabetics with the impaired first phase of insulin secretion in comparison to relatives with autoantibodies but still with normal B-cells function (19±7 vs. 27±8 cells/mm^3, $p<0,005$ and 67±35 vs. 98±29 cells/mm^3, $p<0,01$), patients with clinical diabetes and healthy controls. **Conclusions:** Our study suggests that the γδ T cells could play an important role in the development of IDDM. It is possible that their levels in the peripheral blood could be an additional marker of preclinical detection of the disease, but further prospective studies in high risk of IDDM subjects are needed.

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RAISED ANTIBODIES TO β-CASEIN IN TYPE 1 DIABETES AND COELIAC DISEASE BUT NOT IN OTHER AUTOIMMUNE DISEASES

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Since the association between Type 1 diabetes and cow's milk consumption, enhanced immune reactivity to different cow's milk proteins was reported in patients with Type 1 diabetes. **Aims:** To evaluate the antibody response to one cow's milk protein of particular interest such as β-casein. **Materials and Methods:** β-casein antibodies were measured in a large population of subjects including 71 patients with Type 1 diabetes of recent onset, 24 1st degree relatives of Type 1 diabetic probands, 100 patients with latent autoimmune diabetes in adults (LADA), 50 patients with autoimmune thyroid disease, 50 patients with Type 2 diabetes, 24 patients with multiple sclerosis, 33 patients with coeliac disease, 48 diabetic pregnant women and 97 healthy subjects. Antibodies to bovine β-casein were measured by an immunoenzimatic assay (ELISA) in a total of 497 sera. Furthermore, the binding to β-casein has been also evaluated by immunoblotting analysis. **Results:** Significantly increased levels of IgG antibodies to β-casein were found in patients with Type 1 diabetes and in patients with coeliac disease compared to healthy subjects ($p=0.019$ and $p=0.023$, respectively). Patients with other disease conditions showed significantly low levels of β-casein antibodies compared to healthy subjects and/or patients with Type 1 diabetes matched for age and sex. **Conclusion:** These results demonstrate that antibody levels to bovine β-casein are higher in patients with Type 1 diabetes and coeliac disease compared to normal subjects and/or other autoimmune diseases suggesting that the immune response to β-casein may reflect intestinal autoimmune disorders common to Type 1 diabetes and coeliac disease.

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LEVELS OF ADHESION MOLECULES IN NEW ONSET TYPE 1 DIABETIC PATIENTS.

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Aims: Levels of soluble forms of adhesion molecules have been found increased in patients with onset autoimmune disease, such as Graves' disease. In new onset type 1 diabetic patients, this fact was suggested in one work. So the aim of the study was to investigate levels of InterCellular Adhesion Molecule-1 (sICAM-1), Vascular Cell Adhesion Molecule-1 (sVCAM-1) and L-selectin (sL-sel), in new onset type 1 diabetic patients, with or without Graves'disease. **Materials and Methods:** Sera were collected from 30 healthy controls and from 59 patients with new onset type 1 diabetes alone (n=47) or associated with Graves' disease (n=12). No patient had vascular complication. Levels of soluble adhesion molecules were determined by ELISA method (Immunotech, Marseille, France). **Results:** In patients with type 1 diabetes alone, no significantly increased levels of adhesion molecules were found (sICAM-1: 512 ± 158 vs 523 ± 175 ng/ml; sVCAM-1: 594 ± 201 vs 644 ± 104 ng/ml; sL-sel: 1655 ± 342 vs 1635 ± 262 ng/ml). By contrast, in patients with type 1 diabetes and Graves' disease, levels of sICAM-1 were elevated (sICAM-1: 740 ± 228 vs 523 ± 175 ng/ml-p=0.001 with Mann-Whitney test; sVCAM-1: 655 ± 181 vs 644 ± 104 ng/ml; sL-sel: 1811 ± 262 vs 1635 ± 262 ng/ml). **Conclusions:** We do not confirm that levels of soluble adhesion molecules increased at onset of type 1 diabetes alone. By contrast, patients with type 1 diabetes and Graves' disease have increased levels of sICAM-1. It may be secondary in these last patients to the Graves' disease autoimmune process, as already reported.

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T HELPER 1 PROFILE OF RECENTLY ACTIVATED CIRCULATING T CELLS IN TYPE 1 DIABETES.

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Aim: Studies of both serum and stimulated peripheral blood mononuclear cells (PBMC) have shown increased IFN-γ and TNF-α levels in type 1 diabetes. These findings however, do not clearly differentiate active insulitis from longstanding diabetes and there remains a pressing need for a marker of ongoing autoimmune activity. Since T cells express the activation marker HLA-DR on their surface for about 2 weeks only following encounter with antigen, this population may reflect the phenotype of cells recently involved in the autoimmune process. We have therefore taken the novel approach of isolating this small population (2% of PBMC) and analysing their cytokine profile. **Methods**: PBMC were isolated from 11 type 1 diabetic patients within 6 weeks of diagnosis, and 6 age matched healthy controls. T cells were separated to 95% purity by negative selection using magnetically labelled antibodies (MACS). The DR+ T cells were then enriched 10-15 fold by a positive immunomagnetic separation. The DR- and DR+ T cells fractions obtained were cultured with PMA/Ionomycin for 24 hours.and IFN-γ and IL-2 concentrations measured in the cell supernatants by specific capture ELISA **Results:** In diabetic patients, there was a 3.5 fold increase in IFN-γ secretion in the DR+ population compared with the DR- cells (Median = 494 v 1240 pg/ml $p = 0.03$). There was also a 4 fold increase in basal IL-2 secretion in DR+ cells (median = 11 v 44 pg/ml, $p=0.009$). In healthy controls however, IFN-γ and IL-2 production did not differ significantly between DR+ and DR- T cells. **Conclusion.:** Activated (HLA-DR+) T cells in recent onset type 1 diabetes have an exaggerated Th1 phenotype with increased levels of IFN-γ, and IL-2 production. These findings suggest that cytokine phenotyping of the circulating recently activated (DR+) T cell population may provide a new approach to monitoring disease activity in type 1 diabetes.

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SOLUBLE INTERLEUKIN-2 RECEPTOR IN PREDIABETES, IN OVERT DIABETES TYPE 1 AND DIABETES TYPE 2

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Aims: The purpose of this study was the evaluation of the relationship between ICA occurrence and the level of soluble interleukin-2 (sIL-2r) receptor in the blood serum of patients: in prediabetic period, at the onset of diabetes type 1 and at the onset of diabetes type 2. **Materials and Methods:** The subjects studied were: 35 prediabetic children (mean age 12.5 ± 4.6 yrs), 40 children and 18 adults at the onset of diabetes type 1 (mean age respectively 10.0 ± 4.0 yrs and 32.3 ± 17.5 yrs) and 27 adults with newly diagnosed diabetes type 2 (mean age 45.9 ± 17.3 yrs). All prediabetics and all patients with type 1 diabetes were ICA positive. The control group consisted of 30 healthy individuals. ICA were determined by indirect immunofluorescence on cryostat sections of pancreas blood group type 0. The results were expressed in Juvenile Diabetes Foundation (JDF) units. A positive result was defined as 5 JDF units or greater. Circulating sIL-2r in blood serum was quantified using an immunoenzymatic method. **Results:** Levels of sIL-2r exceeding the highest normal value were found in 21/35 (60%; p<0,0001) prediabetic children, 17/40 (42,5%; p<0,001) diabetic children, 7/18 (38,9%; p<0,003) diabetic adults with type 1 diabetes, 4/27 (14,8%; NS) patients with type 2 diabetes and in 1/30 (3.3%; NS) of the control group. Levels of sIL-2r were higher in the prediabetic group (median: 1224 pg/ml; p<0,008) and in diabetic children (median: 972 pg/ml; p<0,05) compared to the control group (median: 784 pg/ml). No statistical differences were observed in levels of sIL-2r between all adult diabetic patients and the control group. **Conclusions:** The study's results point out that levels of sIL-2r increased in prediabetics and adults and children with type 1 diabetes thus indicating that sIL-2r may be implicated in pathogenesis of diabetes type 1.

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REGULATION OF IL-10 RELEASE FROM PERIPHERAL BLOOD CELLS BY EPINEPHRINE IS DISTURBED IN DM TYPE 1

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Interleukin-10 (IL-10) is an immosuppressive cytokine which may play a crucial role in development of autoimmune diabetes of NOD mice. Only few data on cytokine regulation in humans with DM type 1 exist. Recently, we reported on diminished IL-10 release during hypoglycemia in patients with DM type 1 compared to healthy controls. In addition, release of epinephrine, a known stimulus of IL-10, was reduced. **Aims:** To study the functional capacity of immunocompetent peripheral blood cells (PBC) to release IL-10 and the pro-inflammatory cytokine TNF-α and to investigate cytokine regulation by epinephrine. **Methods:** Whole blood from 20 patients with DM type 1 (age: 35.4±13 years, 8.3±9 years duration of DM) and 24 healthy controls (age 36 8±13 years) was incubated with LPS (10ng/ml) with or without epinephrine (0.1, 1 and 10ng/ml) for 4h. IL-10 and TNF-α were determined in the supernatant by ELISA. **Results:** LPS-stimulated IL-10 (4.6±0.8 vs 7.0±2.4 pg/1mio leuco) and TNF-α release (2.7±0.3 vs. 2.0±0.2ng/1mio leuco) did not differ between both groups. Epi (10ng/ml) enhanced IL-10 by 111±20% in controls which was significantly (p<0.001) higher than in DM1 (61±12%). Epi dose-dependently inhibited TNF-α release in DM1 and controls by 49% and 57% (n.s.). **Conclusions:** The functional capacity of PBC to secrete IL-10 in response to epinephrine was reduced in DM1 whereas TNF-α release was normal. These data are suggestive of a disease-inherent defect of immunocompetent cells in DM1. The lack of IL-10 response to epinephrine may have implications in situations with increased sympathetic activity.

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ADVANCED GLYCATION END PRODUCT-INDUCED TNF-α AND IL-6 PRODUCTION BY PERIPHERAL LYMPHOCYTES OF DIABETIC PATEINTS

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Aim: To investigate whether advanced glycation end product (AGE) stimulates human lymphocytes to produce cytokines and the immune response to AGE stimulation is the same in diabetics and normal subjects since AGE,easily formed in diabetes, might become autoantigenic through modification of protein structure. **Materials and Methods:** Venous blood was collected from 55 normal subjects and 50 diabetic pateints. The blood sample was mixed in 1640 culture medium with 10 μg/ml PHA (Sigma) or 20 μg/ml AGE respectively for 24 hours incubation. TNF-α and IL-6 were determined by ELISA test kit. **Results:** After 24 hours incubation, AGE-induced TNF-α production in diabetic patients and normal subjects was 473.20 ± 66.93 pg/ml vs 254.62 ± 35.14 pg/ml ($P < 0.02$). IL-6 production in diabetes and normal groups was 2400.0± 399.0 pg/ml vs 1983.9±294.8 pg/ml ($P < 0.02$). There was no relation of higher cytokine production to sex, BMI, course of diabetes and blood glucose level ($\gamma = 0 - 0.21$, $P>0.05$). PHA-induced TNF-α production was also higher in diabetes than that in normal control (1313.26±162.6 pg/ml vs 381.04±28.98 pg/ml , $P < 0.002$). There was no difference of PHA stimulated IL-6 production in these two groups. **Conclusions:** The above results demonstrated that 1) AGE can stimulate human lymphocytes to produce TNF-α and IL-6 ; 2) The immune response to AGE and PHA was more sensitive in diabetics , at least partially, than that in normal subjects; 3) It seems that the sensitive response to AGE stimulation has no relation to sex, BMI, course of diabetes and glycemia in tested individual. Our findings imply the possibility that immune response to AGE of diabetic individual can be studied by this method. The sensitive reponse to AGE stimulation in diabetes may be an independent risk factor involved in diabetic complications .

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ROLE OF L-SELECTIN, VLA4, LEUKOSIALIN AND BETA1 INTEGRIN IN DIFFERENT STAGES OF AUTOIMMUNE TYPE 1 DIABETES

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Aims: In this study we investigated the role of adhesion molecules belonging to selectin and integrin families expressed by lymphocytes in different stages of autoimmune Type 1 diabetes. Materials and Methods: Four different stages of diabetes were studied. While preclinical stage group (G1) consisted of first degree relatives of Type 1 diabetics (n=11) having ICA≥20 JDF u. and acute insulin response in IVGTT below the 3. percentile of normals, early clinical group (G2) was composed of newly diagnosed diabetics (n=14). Diabetics diagnosed between 6-12 months (n=15) formed the clinical stage group (G3) and Type 1 diabetics having a diabetic period of 1-5 years (n=14) constituted the advanced clinical stage group (G4). Control group consisted of 14 healthy, ICA (-) subjects(G5). Flow cytometric analysis was used for measurement of CD62L (L-selectin), CD49d (VLA 4), CD43 (Leukosialin) and CD29 (Beta 1 integrin). Results: L-selectin was significantly increased in G1 and G3 ($p<0.05$) and in G2 and G4 compared to G5 ($p<0.01$). VLA 4 expression was found to be significantly increased in all diabetic groups ($p<0.001$ in G1,G3 and $p<0.01$ in G2,G4). Leukosialin showed no significant alteration in any group ($p>0.05$). All stages were found to have significantly high levels of Beta 1 integrin ($p<0.001$ in G1, G2, G4 and $p<0.05$ in G3). Conclusions: Our results indicate that adherence and then rolling of lymphocytes on endothelial cells may play a role in pre- and early clinical stages of Type 1 diabetes, but in all other stages a relatively strong adhesion was observed.

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EXPRESSION OF NEUTROPHIL ADHESION MOLECULES IN EARLY PREGNANCY OF DIABETIC MOTHERS.

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The effective functions of human peripheral blood neutrophil are modulated by regulatory molecules depending on receptor agonists and adhesion molecules. **Aim of study**: Evaluation of percentage of neutrophil carrying some adhesion molecules in early pregnancy of diabetic mothers as well as their expression. **Material:** 9 pregnant diabetic women demonstrating good glycemic control and without major complications before pregnancy were studied at 7-13 weeks of first gestation, 6 healthy pregnant women match for weeks of gestation, age and parity ; 9 healthy non-diabetic women; 12 diabetic, non-pregnant women, were also studied. **Methods:** Peripheral blood ~~neutrophil adhesion molecules [CD18, CD11b, CD62L and CD54] were evaluated~~ both MFI expression and percentage when resting and activated by 30min., fLMP preincubation using monoclonal antibodies anti-CD11b and anti-CD62L (Becton-Dickinson) employing Flow Cytometer FACSCalibur with computer program CELLquest. **Results:** Expression of CD11b receptor on leukocyte of diabetic pregnant mothers was higher both spontaneous and after stimulation by fLMP in compare to group of healthy women and diabetic nonpregnant one. The significant difference in CD62L MFI expression was noticed both for resting and activated neutrophils in healthy pregnant women group when compare to healthy non-pregnant and other studied groups. A decrease in percentage of CD62L/fLMP molecule was noticed in all groups when compare to healthy nonpregnant. MFI expression of CD54 was lower in pregnant diabetic women versus diabetic non pregnant women. Although there was an increase in expression of CD54 on resting neutrophils of diabetic nonpregnant when compared with healthy. We did not noticed significant difference in expression of maternal CD18 in peripheral blood neutrophils in early pregnancy, in all studied groups. **Conclusions:** diabetes type 1 with good glycemic control and pregnancy changed the percentage and MFI of neutrophil bearing adhesion molecules.

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DIFFERENTIAL TUMOR NECROSIS FACTOR AND INTERLEUKIN-6 PRODUCTION IN DIABETIC PATIENTS WITH DIABETIC NEUROPATHY TREATED WITH A-LIPONACID

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Aims:

Administration of tumor necrosis factor (TNF-a) increases whole body glucose kinetics and stimulates in vivo glucose uptake by several tissues.As it is known that blood glucose changes after a-Liponacid infusion we examined serum TNF-a and (IL-6) Interleukin-6 activity in treated diabetic patients with peripheral neuropathy.

Materials and Methods:

28(16) diabetic patients with peripheral neuropathy were treated with 600mg a-Liponacid in 250 ml 0,9% NaCl over a period of 14 days.Cytokine levels were measured before and after intravenous infusion.All cytokines were measured by ELISA (Medgenix).Blood glucose was measured by Hemocue before starting infusion and when stopped.The differences in neuropathy symptoms were signed in a neuropathy score system.

Results:

67,85% (19/28) of the patients showed elevated TNF-a serum levels after a-Liponacid infusion,28,57%(9/28)had lower TNF-a levels than before treatment.75(12/16)of the patients had decreased serum IL-6 levels after a-Liponacid injection,25% (4/16) answered without any reaction.In patients pretreated with immunsuppressive drugs like corticoids and Azulfidines changes in cytokine system were not seen.In all cases without pretreatment with immunsuppressive substances IL-6 decreased significantly while TNF-a levels and blood glucose increased significantly (pless than 0,05).

Conclusions:

Our findings suggest that a-Liponacid as a component of the Pyruvat-Dehydrogenase complex plays an important role in stimulation and modulation of the cytokine system. The ability to induce these changes is stopped when immunsuppressive substances possibly block this Multienzyme-system.The beneficial effect in neuropathy symptoms could be explained by decreasing systematic IL-6 serum levels.

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MANIFESTATION OF TYPE I DIABETES MELLITUS IN A PATIENT WITH HEPATITIS C DURING THERAPY WITH α-INTERFERON AND RIBAVIRIN

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Aims: Some cases of Type I diabetes during therapy with alpha-interferon and ribavirin in chronic hepatitis C have been reported. We report on a patient, who developed Type 1 diabetes under continued therapy with α-interferon and ribavirin. **Materials and Methods**: A male patient (age 35 yrs, BMI 27.1 kg/m^2, HCV-RNA-positive; genotype 2a; AST 43 U/L, ALT 136 U/L, y-GT 37 U/L, AP 95 U/L) was treated according to a study protocol with alpha-interferon (3 million IU/d s.c.) and ribavirin (1200 mg/d). After four months of treatment the patient developed polyuria, polydipsia and a weight loss of 8 kg. In the basal state and after administration of 1 mg glucagon i.v., we determined glucose, insulin and C-peptide after 3, 6 and 10 min. In blood samples drawn before and after therapy we measured GAD-antibodies. **Results**: At diabetes onset, the plasma glucose level was 457 mg/dl, the patient developed ketonuria, and the BMI had decreased to 24.3 kg/m^2. Glucagon stimulation test: glucose 353, 296, 348, 374 mg/dl, insulin 1.8, 0.2, 2.0, 2.7mU/l, C-peptide 0.009, 0.13, 0.05 and 0.03 nmol/l (showing a significantly reduced insulin response). Before therapy, GAD-antibodies were negative in one blood sample. At time of diabetes onset, GAD-antibodies became positive (466 ng/ml, reference < 32 ng/ml). The patient was treated with intensified insulin therapy, the therapy with alpha-interferon and ribavirin was stopped (HCV-RNA remained negative, AST and ALT were of normal value until 6 months after stopping antiviral therapy). **Conclusions**: Of special interest in this case is the short duration (16 weeks) between being GAD-antibodies negative before therapy and diabetes onset with positive GAD-antibodies. Whether there really is an increased incidence of Type I-diabetes during alpha-interferon and ribavirin therapy is not yet clear.

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SUPPRESSION OF AUTOIMMUNE DIABETES BY CHOLERA TOXIN B CHAIN: ROLE OF INNATE IMMUNITY

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Aims: The cholera toxin B chain (CTB) has been reported to suppress T cell dependent autoimmune diseases including autoimmune diabetes of NOD mice. We have tested the hypothesis that CTB exerts much of its immunomodulatory activity by targeting the innate immune system. Materials and Methods: Human monocytes Mono Mac 6 were exposed to CTB and subsequently tested for proinflammatory immunoreactivity in response to challenge with endotoxin (LPS from E. coli). Levels of TNFα in culture supernatants were determined by sandwich ELISA. Results: Incubation of monocytes with CTB (10 µg/ml) suppressed a later proinflammatory response to LPS within 5 h as demonstrated by suppressed production of TNFα ($p<0.01$), while IL-10 remained inducible. Control experiments excluded a role of possible contamination of CTB by endotoxin or the intact cholera toxin. Tolerance induction by CTB was dose and time dependent and could be prevented by the addition of antibodies to IL-10 and TGFβ. IFNγ also antagonized CTB actions on macrophages. In contrast to desensitization by low doses of LPS, tolerance induction by CTB occurred "silently", i.e. in the absence of a measurable proinflammatory response. Conclusions: In view of the potent instructive role of the innate immune system on T cell responses these findings are important in understanding how CTB prevents the development of autoimmune diabetes and improves tolerance to islet autoantigens.

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PERFORIN PLAYS A BETA-CELL CYTOTOXIC ROLE ON THE PATHOGENESIS OF NIDDM INDUCED BY VIRUS INFECTION

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Aims; The NDK25 variants of Encephalomyocarditis (EMC) virus can induce NIDDM in RAG-2 knockout (KO) mice that lack both T- and B-lymphocytes, suggesting mature lymphocytes play a protective role. Whereas, the pathogenic mechanisms remain ill-defined. This study was initiated to elucidate the role of perforin on this model. **Materials and Methods;** RAG-2KO mice, perforin KO mice (PfpKO), RAG-2 and perforin knockout (DoubleKO) mice (n=6-8) were used. Wild type of male 129/Sv mice and C57BL/6 mice were used as control (n=6-8). The NDK25 variants were administrated intraperitoneally at 200 pfu/mouse, and OGTT was performed before and 1 week after infection. **Results;** OGTT showed that blood glucose levels of RAG-2KO after glucose load were significantly higher than those of control mice. Whereas, there were no significant difference of blood glucose levels during OGTT among PfpKO, DoubleKO mice, and control mice. The insulin contents of pancreas in RAG-2KO were 20 $\pm 14\,\mu$g/g of pancreas, that were significantly lower than those in the other group. In contrast, there were no significant difference of the glucagon contents of the pancreas among these four group. In histology, mild mononuclear cell infiltration was observed in the islets of pancreas from each group. In contrast, atrophic islets were observed in RAG-2KO mice. **Conclusions;** Perforin can play a beta-cell cytotoxic role on the pathogenesis of virus-induced NIDDM in the absence of mature lymphocytes.

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EFFECT OF TROGLITAZONE ON CYTOKINE EXPRESSION OF T-CELLS FROM NOD MICE

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$CD4^+$ T-cells can be differentiated by their cytokine profiles in Th1-cells producing IFN-γ and IL-2, and Th2-cells producing IL-4 and IL-10. Th1- and Th2-immune responses are thought to be involved in the pathogenesis of certain autoimmune diseases. Thus, in type 1 diabetes the switch from benign to ß-cell destructive insulitis is accompanied by a shift from Th2- to Th1-cytokines, potentially opening new strategies for prevention of diabetes. **Aims:** This study examined, therefore, whether the prevention of diabetes by troglitazone (TGZ) in NOD mice is related to changes of the T-cell cytokine production profile. **Material and Methods:** Splenocytes from non-diabetic NOD mice treated with TGZ (n=16) or placebo (n=7) for 35 weeks were stimulated in vitro with phorbol ester and ionomycin in the presence of brefeldin A. The permeabilised cells were double-stained by immunofluorescence for one of the surface markers CD4 or CD8, and for one of the cytokines IL-2, IFN-γ, IL-4 and IL-10. The percentage of cytokine-positive T cells and the mean fluorescence intensity (MFI, relative amount of cytokine produced per cell) were determined by flow cytometry. **Results:** The percentage of $CD4^+$ or of $CD8^+$ splenocytes expressing either IL-4 and IL-10 or IL-2 and IFN-γ was comparable between TGZ- and placebo-treated mice (Table). Analysis of T-cells revealed two cell clusters with different IFN-γ expression (MFI±SD: 27.0±7.7 vs. 44.6±8.1; $p<0.05$). 75% (12/16) of TGZ-treated and 42.9% (3/7) of placebo-treated mice had T-cells with IFN-γ MFI level of 27.0±7.7 whereas 25% (4/16) of TGZ- and 57.1 % (4/7) of placebo-treated mice had $IFN\text{-}\gamma^+$ T-cells within the lower MFI cell cluster.

Table. Cytokine-expression of $CD4^+$ and $CD8^+$ T-cells (% double-positive cells ± SD)

	CD4				CD8			
Cytokines	IL-2	IFN-γ	IL-4	IL-10	IL-2	IFN-γ	IL-4	IL-10
TGZ	23.1±6.3	15.4±5.1	2.0±3.2	1.5±1.4	7.3±5.7	18.4±6.2	2.7±5.2	1.0±2.0
Placebo	27.2±9.1	20.4±7.2	1.9±1.3	2.0±1.9	10.3±3.4	17.5±8.0	0.3±0.5	1.4±1.1

Conclusions. The mean percentage of T-cells expressing either IL-4 and IL-10 or IL-2 and IFN-γ was unaffected by TGZ. However, TGZ appears to modulated the intracellular level of IFN-γ expression. Diabetes prevention by TGZ in NOD mice might be mediated by the down-regulation of IFN-γ.

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CELLULAR AUTOIMMUNITY TO PHOGRIN IN THE NOD MOUSE

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The structurally-related protein tyrosine phosphatases IA-2 and phogrin are major targets of humoral autoimmunity in type 1 diabetes in Man however their involvement in the disease in experimental disease models (NOD mouse and BB rat) is disputed. In this study we have evaluated cell-mediated autoimmunity to recombinant phogrin in the NOD mouse. Mesenteric lymphnode cells of young NOD females exhibited a spontaneous T cell proliferative response to phogrin. Immunization of NOD mice with phogrin resulted in a vigorous T cell proliferative response which was antigen specific and cross reactive on islet cells. Limiting dilution yielded 10 T cell clones which reacted to phogrin and three of these reacted with crude granule preparation of rat insulinoma tissue consistent with the documented localization of the antigen. Two of these were capable of destruction of rat islet grafts placed into NOD/scid recipients. All of the phogrin specific T cell clones were CD4 positive by FACS analysis and were TH1-like as defined by their cytokine production profile as analyzed by ELISA. A series of truncated recombinant proteins generated from the phogrin C terminal molecule has been used to map the epitopes recognized by phogrin -specific T cell clones of diabetogenic potential. These results demonstrate that cellular autoreactivity to phogrin arises in the islet during the course of the spontaneous disease and phogrin-specific T cells are capable of destruction of islet cells in vivo. Because of their close structural relationship, further delineation of T cell epitopes shared between IA2 and phogrin will facilitate analysis of the role determinant spreading in the progression of type 1 diabetes.

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FAS (CD95/APO-1) EXPRESSION IN PANCREATIC ISLETS OF DIABETES-PRONE BB RATS

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Aims: Interleukin-1beta (IL-1β) has been shown to induce destruction of pancreatic islet beta-cells and to upregulate Fas in mouse and human islets. We investigated the possible involvement of Fas in IL-1β-induced stress response of pancreatic islets from diabetes-prone BB rats. **Materials and Methods:** After preculture for 3d and exposure to IL-1β (10 U/ml) for 24h or not (control) isolated pancreatic islets were used for functional tests and estimation of spontaneous ^{51}Cr-release. Surface antigen expression was measured on single islet cells by FACS analysis using monoclonal antibodies: MHC class I (OX 18), MHC class II (OX 6), ICAM-1 (1A29), beta cells (K14D10). Islet proteins were separated by SDS-PAGE followed by immunoblotting with C92F3A-5 (HSP 70) and a polyclonal antibody which recognizes Fas. **Results:** IL-1β decreased islet insulin content ($p < 0.001$) and reduced insulin release in response to 20 mmol/l glucose ($p < 0.001$) compared to control islets while no membrane alterations were detectable as measured by spontaneous ^{51}Cr-release. After IL-1β treatment beta cell number was reduced (90.2±0.6% vs. 84.6±1.6%) and ICAM-1$^+$ beta cells (7.4±1.0% vs. 87.5±1.9%) and ICAM-1 antigen density (3.9±0.7logU vs. 15.8±2.3logU) on beta cells were increased while the expression of MHC class I and II was not changed. Whereas HSP 70 only in IL-1β treated islets was detectable, expression of Fas was similarly observed in both control and cytokine-treated islets. **Conclusions:** Our results suggest that in response to IL-1β protective mechanisms are activated in pancreatic islets. But our results do not support the hypothesis of Fas-mediated apoptosis in pancreatic beta cells from diabetes-prone BB rats because IL-1β induced stress response was not accompanied by upregulation of Fas.

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THE LEVELS OF SOLUBLE CYTOKINES IN SERUM CORRELATE WITH CYTOKINE mRNA LEVELS IN PANCREAS AND INSULITIS PROGRESSION IN NOD MICE

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Aim: The development of destructive insulitis and autoimmune diabetes in NOD mice correlates with in-situ expression of Th1 cytokines, whereas non-destructive insulitis is associated with Th2 cytokines. The goal of this study was to determine whether cytokines in mouse serum might correlate with the quality of insulitis and therefore would provide a marker for monitoring disease activity.

Material and Methods: Groups of non-diabetic NOD mice (5-14 weeks old), diabetic NOD mice (14 weeks of age) and age matched C57/BL16 mice were analyzed. The pancreas was removed for histology and RNA extraction, and serum was obtained from blood samples. The cytokines Interferon gamma (Th1) and Interleukin 10 (Th2) were determined from pancreatic mRNA by RT-PCR and in the serum of the same animals by sandwich ELISA.

Results: IFNg mRNA levels were elevated in the pancreas of diabetic animals compared to non-diabetic NOD mice, whereas IL10 message was decreased in diabetic animals. Levels of soluble cytokines in serum correlated with that of cytokine mRNA in pancreas. The ratio of IFN-g to IL-10 in diabetic animals differed significantly ($p<0.0001$) from that of non-diabetic NOD mice.

Conclusion: In an animal model of type 1 diabetes circulating cytokines in serum reflect disease activity in the pancreas, as determined by cytokine gene expression pattern. Serum cytokine patterns may allow monitoring of disease activity in human patients.

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PRIMARY NONFUNCTION OF ISLET XENOGRAFTS IN SPONTANEOUSLY DIABETIC AUTOIMMUNE NOD MICE: CORRELATION WITH ELEVATED NON-T CELL CYTOKINES IN THE GRAFTS.

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Primary nonfunction (PNF) and early graft destruction are obstacles for the application of islet xenotransplantation in autoimmune type 1 diabetes. **The first aim** of this study was to investigate the role of autoimmune disease recurrence in PNF and xenograft survival in autoimmune NOD mice after rat islet transplantation. **The second aim** was to examine the mediators of this early islet destruction. **Results**: Spontaneously diabetic autoimmune NOD mice had a shorter islet xenograft survival (5.4 ± 2.3 days) compared to chemically diabetic C57Bl/6 or old NOD mice (11.3 ± 1.2 days, $p<0.01$ and 9.7 ± 3.7 days, $p<0.05$). Also a higher incidence in PNF (44%) was noted in these autoimmune diabetic mice compared to non-autoimmune diabetic C57Bl/6 or old NOD mice (10% and 29%). Analysis of cytokine mRNA levels locally in the xenografts showed clearly higher IL-1α and TNF-α levels in spontaneously diabetic autoimmune NOD mice 8 hours posttransplantation, rapidly decreasing after 16 hours. In the islet xenografts of the non-autoimmune diabetic mice (chemically diabetic C57Bl/6 or old NOD mice), elevated levels of IL-1α and TNF-α were only noted at 16 hours after transplantation. Another difference between autoimmune and non-autoimmune mice were the levels of the suppressive cytokine, TGF-β, being clearly higher (2 fold) in the non-autoimmune situation. T-cell cytokines (IL-2, IL-4 and INF-γ) in islet xenografts of both autoimmune and non-autoimmune diabetic mice were absent or similar with background levels in the first 24 hours after transplantation, as were T cells on immunohistochemistry. **In conclusion,** these data suggest a role for the autoimmune memory in the occurrence of PNF and islet xenograft destruction in the spontaneously diabetic autoimmune NOD mice. The destruction of islets seems however to be mediated primarily by non-T cell types such as macrophages.

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THE HU-PBL-SCID MOUSE AS MODEL FOR ISLET XENOTRANSPLANTATION.

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Type 1 diabetes is the result of an autoimmune attack against the pancreatic β cells, causing a destruction of the target tissue. Transplantation of xenogeneic islets can be a possible treatment. SCID mice, characterized by a severe immunodeficiency and the acceptance of allo- and xenogeneic grafts may be a possible tool for research in this field. **The aim of the study** was to develop an *in vivo* animal model in which immunological interactions between the human immune system and transplanted xenogeneic islets could be studied. **Methods**: 150 rat islets were grafted under the kidney capsule of alloxan diabetic SCID mice before reconstitution with 50×10^6 huPBL or with 30×10^6 huPBL followed by a booster of 10×10^6 aCD3 pre-activated huPBL. Glycemia was assessed every two days. Postglucose (2g/kg BW) glycemia, plasma rat C-peptide and insulin were measured weekly. At the end of the study, grafts were removed for histology and immunohistochemistry (IHC). **Results**: Although all groups remained normoglycemic until the end of the study, histology and IHC of the grafts revealed a severe human CD45$^+$ cell infiltration in the grafts of the boostered mice compared to no infiltration in the unreconstituted group or minor infiltrates in the unboostered group. After glucose challenge, unreconstituted mice remained normoglycemic (84 ± 16 mg/dl) with -compared to initial levels- elevated C-peptide (194 ± 110% of initial levels) and insulin (187 ± 110% of initial levels) measurements 3 weeks post sham reconstitution. The unboostered group had at this time point a slight decrease in both insulin (64 ± 54%) and C-peptide (73 ± 57%) production, however still maintaining normoglycemia (104 ± 55 mg/dl) after glucose challenge. A completely impaired islet xenograft function was demonstrated in the boostered group as suggested by hyperglycemia (323 ± 146 mg/dl) and a significantly reduced insulin (23 ± 12%, $p<0.05$) and C-peptide secretion (11 ± 8%, $p<0.05$) after glucose challenge 3 weeks post reconstitution. These observations lead to the **conclusion** that pre-activation of human lymphocytes is essential in graft destruction in the hu-PBL-SCID mouse model.

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INFLUENCE OF FREE AND ENCAPSULATED ISLETS XENOTRANSPLANTATION ON THE CHEMOTAXIS OF PERITONEAL MACROPHAGES :

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In vitro, we have shown that pancreatic islets stimulated macrophage migration by the release of immunological specific proteins partly retained by macroencapsulation. **The aim** of these present work was to study the influence of the transplantation of free and encapsulated (AN69 membrane, Hospal, France) rat islets on the chemotaxis of peritoneal murine macrophages. **Materials and methods:** 50 free and encapsulated rat islets cultured for 24 hours were transplanted in the peritoneal cavity of healthy mice (n=6). Untreated mice, mice operated but not transplanted and mice transplanted with an empty macroencapsulation device served as controls (n=5). After 3 days transplantation, peritoneal murine macrophages were collected and tested for their chemotaxis toward formyl-Methionyl-Leucyl-Phenylalanine (fMLP), and culture medium conditioned for 4 days by free rat islets isolated from the same rat donor as grafted islets. The macrophage chemotactic index was defined as the ratio: number of cells attracted by the conditioned culture medium (or fMLP) / number of cells attracted by the culture medium. **Results:** In response to fMLP, the chemotactic indexes of macrophages from mice transplanted with free and encapsulated islets were respectively 7.66±1.86 and 8.42±2.22. These values were significantly increased compared to those obtained with macrophages from untreated mice (2.90±0.37; $p<0.01$). Transplantation of an empty device or surgery also induced an increase in macrophage migration toward fMLP (5.60±1.20 and 4.46±1.30) but significantly lower than that observed with macrophages from mice transplanted with islets ($p<0.05$). Toward culture medium conditioned by free islets, transplanted encapsulated islets failed to enhance macrophage chemotaxis (1.98±0.40) compared to transplanted free islets (5.62±1.96; $p<0.01$). Macrophage migration from operated mice and mice transplanted with an empty device was similar to that of untreated mice. **Conclusions:** Islet encapsulation decreased the specific chemotactic reactivity of peritoneal macrophages induced by free islet transplantation.

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TOLERANCE INDUCTION TO ISLET ALLOGRAFTS

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Mycophenolate mofetil (MMF), a selective inhibitor of T and B cell proliferation currently in clinical use for the prevention of allograft rejection, has been suggested to induce donor-specific tolerance to islet allografts in adult mice. 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], the active form of vitamin D_3, is an immunomodulator able to prolong heart allografts and to ameliorate autoimmune diseases, including autoimmune diabetes, which inhibits the ability of APC to induce T-cell activation via inhibition of IL-12 production and downregulation of class II MHC and costimulatory molecule expression. **Aims:** to analyze the capacity of MMF and $1,25(OH)_2D_3$ administered alone or in combination to induce tolerance to pancreatic islet allografts in mice. **Materials and Methods:** 350 B6 mouse pancreatic islets were transplanted under the kidney capsule into fully mismatched BALB/c mice rendered diabetic by streptozotocin administration. Blood glucose levels were serially monitored in the recipients. **Results:** MMF (100 mg/kg po daily from d -1 to 30 after islet transplantation) and $1,25(OH)_2D_3$ (5 μg/kg po 3x/week from d -1 to 30) administered alone were scarcely effective in prolonging islet graft survival. However, 70% of recipient mice treated with both drugs showed long-term (>70 d) graft acceptance and following drug withdrawal all mice remained normoglycemic. Allografts accepted under the cover of MMF and $1,25(OH)_2D_3$ were more resistant to rejection upon challenge with B6 donor spleen cells than grafts accommodated under the cover of MMF alone. Mice still normoglycemic 4 weeks after spleen cell challenge received a non-vascularized heterotopic heart graft from B6 neonates. Mice which did not reject islet tissue even after a spleen cell challenge were also tolerant to a donor-type heart graft, indicating that the combination of MMF and $1,25(OH)_2D_3$ is able to induce an effective donor-specific transplantation tolerance in adult mice. **Conclusions:** these results indicate the possibility to use a combination of MMF $1,25(OH)_2D_3$ and to prolong human islet allografts. This regimen may not only prevent graft rejection and possibly induce tolerance, but also inhibit the autoimmune response.

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Epidemiology of Type 2 Diabetes I

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Characteristics Associated with Early Onset of Type 2 Diabetes in Adults

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Aims: To determine whether adults diagnosed with Type 2 diabetes between age 18-44 years (EarlyDM2) have distinctive baseline risk profiles compared to adults diagnosed ≥45 years (UsualDM2).

Materials and Methods: We studied baseline characteristics among 2,963 adults in a large health maintenance organization diagnosed with Type 2 diabetes between 1996-97 who had measured weight, HbA1c, and blood pressure within 3 months of diagnosis. Electronic medical and laboratory records were used to abstract clinical data. Parametric t-tests and chi-square analyses were used to compare mean and proportional differences, respectively. To determine if significant univariate variables were independently associated with EarlyDM2 and control for other possible confounders, multiple logistic regression was performed for a subset of 692 that responded to a 1997 sociodemographic survey.

Results: Adults with EarlyDM2 were significantly more obese at the time of diagnosis than adults with UsualDM2 (BMI 36.6 vs. 32.3 kg/m^2, $p<0.001$). Glycemic control at diagnosis was not different (HbA1c 7.8 vs. 7.7%). Diastolic blood pressure (Dbp) with EarlyDM2 was slightly higher at diagnosis than UsualDM2 (81 vs. 79 mmHg, $p<0.05$) despite lower systolic (S) bp (130 vs. 136 mmHg, $p<.001$). Higher BMI, higher Dbp, and lower Sbp remained independently associated with EarlyDM2 on multivariate analysis ($p<0.02$). Non-white race and female gender were also associated with EarlyDM2 ($p=0.005$ and 0.04, respectively), but educational level was not. BMI had the strongest association with EarlyDM2 on multivariate analysis (for every kg/m^2 increase in BMI, the odds of EarlyDM2 increased by 8%, $p=0.0002$).

Conclusions: Morbid obesity is a significant predictor of DM2 before age 45 years. Intensified screening for DM2 in very obese young adults should be considered, particularly in ethnic groups with a higher prevalence of DM2 and/or obesity. Further research is needed to determine the effect of increasing obesity to alter the time course of developing DM2 in those that are genetically predisposed.

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EFFECTS OF FAMILIAL HISTORY OF DIABETES ON CLINICAL CHARACTERISTICS OF TYPE 2 DIABETIC PATIENTS.

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Aims: to evaluate if the presence of diabetes in relatives and in parents may influence metabolic control and the presence of chronic complications in type 2 diabetic patients and if there is a possible differential influence of maternal or paternal diabetes on these characteristics. **Material and methods:** all 2113 patients with type 2 diabetes, referring to the Diabetic Clinic of Asti were recruited; these patients represent the 1.6% of the reference population, that is about 80% of diabetic patients of the area. The patients were asked wheather any of their family members had diabetes diagnosed by a physician. BMI, blood pressure, lipid parameters, HbA1c, and C-peptide levels were measured; the patients were yearly screened for the presence of chronic complications. **Results:** 25.5% of patients had a a diabetic mother, while 6.5% had a diabetic father ($p<0.0001$), 21.2% had other relatives with diabetes and 46.7% had no relatives with known diabetes. No differential influence was found between maternal and paternal diabetes, on clinical characteristics of the patients. By comparing the following groups: parental diabetes, diabetes in other relatives, no known diabetes in relatives, we found age, age of diabetes diagnosis, total and LDL-cholesterol and prevalence of hypercholesterolemia and retinopathy as significantly different between the three groups. In a logistic regression model, patients with parental diabetes had significantly lower age (β= -0.030, $p=0.0003$), age of diagnosis of diabetes (β= -0.026, $p=0.0014$), and LDL-cholesterol (β= 0.0068, $p=0.030$) in respect to those without known diabetes in their family. No relevant difference was found between those with other relatives with diabetes and other groups of patients. **Conclusions:** the presence of diabetes in relatives has no role in conditioning clinical characteristics of type 2 diabetes mellitus and we could not find any differential influence between paternal and maternal diabetes. Patients with parental diabetes showed an earlier age of onset of the disease and higher LDL-cholesterol, supporting the hypothesis that genetic component is more important when the disease was diagnosed at an early age and that a complex metabolic syndrome may be inherited.

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PROGRESSIVE WORSENING OF HbA1c IN 10 YEARS OF FOLLOW-UP IN TYPE 2 DIABETES

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UKPDS showed that HbA1c worsened during the ten years of the study irrespective of the therapy used (conventional or intensive); also the body weight worsened, but with an higher increase in the subgroup of patients in intensive treatment. We retrospectively evalued the trend of HbA1c, body weight, BMI and blood pressure (annual mean) in 100 type 2 diabetic patients (age 66.5±10.7 years, mean ± SD, 45% female, duration of diabetes at 1988 7.03±5.41 years), continuative referred to our diabetes care unit at least for 10 years (1988 time 0; 1993 after 5 years; 1998 after 10 years). Statistical analysis has been made with ANOVA. The body weight (time 0: 72.2±13.5 Kg, after 5 years: 73.8±13, after 10 years: 72.2±12) and the BMI (time 0: 27.7±4.79 Kg/m^2, after 5 years: 28.2±4.69, after 10 years: 27.5±4.33) did not show statistical difference. HbA1c (time 0: 7.65±1.26 %, after 5 years: 8.72±1.46, after 10 yeas: 9.07±1.56, p<0.0001, time 0 vs 5 yeras and time 0 vs 10 years) worsened during the years of follow-up, independently from gender, age of the patient, duration of diabetes, body weight, kind of therapy (diet, OHA, insulin) and value at baseline. The blood pressure did not change considering the systolic values (time 0: 146.8±20.8 mm Hg, after 5 years: 145±18.6, after 10 years: 142.2±16.9, n.s.), whereas the diastolic values decreased (time 0: 84.9±10.5 mm Hg, after 5 years: 82.2±10.5, after 10 years: 78.3±9.0, p<0.0001, time 0 and 5 years vs 10 years); we also observed an increase in the number of diabetic patients treated for hypertension (time 0: 21%, after 5 years: 29%, after 10 years: 36%). We evalued also the prevalence of retinal complications, as index of microangiopathy: 10 patients developped background retinopathy and 4 patients proliferative retinopathy; this subjects presented higher values of HbA1c at the baseline (time 0: 8.3±1.7 vs 7.5±1.14%, p=0.0149) compared with those without retinal complications. Our study confirms the UKPDS data about the worsening of HbA1c in type 2 diabetic patients, independently from the therapy. The observation that the body weight did not worsened in our study compared to the UKPDS might be explained with the lower degree of insulin therapy (7% vs 16% and 38%, conventional and intensive therapy respectively) and with the importance gived to the behavioural therapy and to the diet.

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PREDICTIVE RISK FACTORS FOR CORONARY HEART DISEASES (CHD) IN TUNISIAN POPULATION

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Previous studies had shown that about 25% of death in Tunisian adult population were related to cardiovascular diseases. **Aims**: the aim of this study was to identify predictive risk factors for non fatal CHD in Tunisian population. **Materials and Methods:** this study included a cohort of 736 adults (age ≥29 years, sex ratio 0,69) investigated in 1985 and ten years later in 1994 – 1995, with normal ECG at baseline. Weight , height , waist circumferences, systolic and diastolic blood pressures (SBP, DBP), fasting and two hours blood glucose and insulin, glycated haemoglobin and total cholesterol were measured in all subjects at base line. All subjects had ECG at baseline and at the end of the study . ECG were analysed by the same cardiologist. CHD was considered according to the presence of abnormal Q or T waves or ST segments. **Results**: Among the 736 subjects, 90 (12,2%) developed CHD. Subjects who developed CHD were older (50 ± 10,4 vs 46.3 ± 11,3 years, p<0.001). Comparison of age adjusted clinical and biological characteristics recorded at baseline in the two groups showed that subjects with CHD had significantly higher SBP and DBP respectively 142.9 ± 27.4 vs 133.7 ± 20.8 mmHg, p<0.001 and 86.1 ± 13.8 vs 80.9 ± 10.8, p<0.001 .In univariate analyses adjusted OR for CHD were 1.97 with 95 % CI [1.17 , 2.97] for SBP>140 mmHg, 2.19 with 95% CI [1.3, 3.7] for DBP>90 mmHg and 2.74 with CI [1.32 , 5.68] for HbA1C ≥ 6%. Stepwise regression analyses showed that only HbA1C persisted as independent risk factor for CHD OR 2.86 with CI [2.86,6.89]. Hypertension persisted at the limit of significance OR 1.28 [0.99 , 3.18]. In subjects with normal glucose tolerance SBP and DBP persisted significantly associated with CHD, OR were respectively: 1.76 with CI [1.00, 3.12] and 2.19 with CI [1.10, 4.32]. **Conclusion:** strategies of prevention of CHD should insist on subject with blood pressure > 140/90 mmHg and diabetic patients with HbA1C > 6% .

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GLUCOSE TOLERANCE AND ITS RELATION TO REPRODUCTIVE LIFE CHARACTERISTICS IN FEMALE POPULATION OF TURKEY

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Aims: To determine the prevalence of abnormal glucose tolerance (DGT and IGT) among female population of Turkey and to investigate its possible relationships to reproductive life history (RLH). **Material and Methods:** The study included a sample of 13,708 women, aged 20 years and over. Data has been derived from a nation wide field survey which was carried out in 1997. GT was assessed based on WHO 1985 criteria. All attendees received an OGTT after an overnight fast except known diabetics. BP, BMI, W/H were measured. Sociodemographic features and data regarding to RLH (live birth, still birth, total parity, abortion, macrosomic baby, congenital malformations, current pregnancy, menopause, and contraception were obtained through a questionnaire filled by an experienced nurse. **Results:** Overall prevalence of diabetes was 8.9% and of IGT was 8.6%. Frequency of diabetes and IGT according to various RLH characteristics are below:

	Yes		No		ANOVA
	IGT	DGT	IGT	DGT	p-value
Live birth	9.0	9.2	7.1	7.9	0.0001
Still birth	11.4	12.9	8.5	7.9	0.0001
Overall parity	9.0	8.6	7.1	8.0	0.0001
Abortion	10.4	11.0	8.3	7.7	0.0001
Cong. Malformation	10.3	9.7	8.9	8.6	NS
Macrosomia	9.8	8.6	8.8	8.5	NS
Current pregnancy	5.8	3.3	8.8	8.3	NS
Menopause	12.8	17.0	6.5	4.0	0.0001

Multiple Logistic Regression Model showed that age, parity, menopause, BMI and BP are independent predictors of abnormal GT (p<0.0001, <0.002, <0.05, <0.05 and <0.01). **Conclusions:** RLH as well as general female health have a strong influence on GT in Turkey.

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LEPTIN LEVEL IS NOT ASSOCIATED WITH CARDIOVASCULAR MORTALITY: THE HOORN STUDY

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Aims - Several cross-sectional epidemiological studies have shown associations between high leptin level and components of the insulin resistance syndrome, which cannot completely be explained by its strong correlation with body fat. Possibly, leptin is a cardiovascular risk indicator. So far, long-term prospective studies on the predictive value of leptin for (cardiovascular) mortality have been lacking.

Materials and Methods - In the Hoorn Study, a population-based cohort of 2345 subjects, aged 50-75 years, the associations between fasting leptin levels (baseline examinations 1989-1990) and cause-specific 9-year mortality were studied. Subjects were grouped into quintiles of leptin level. Cox' proportional hazard analysis was used to estimate relative risks (hazard ratio's).

Results - Table 1. Age- and sex adjusted relative risk (95% CI) of cause-specific 9-year mortality in quintiles of baseline leptin, relative to the lowest quintile (< 2.6 μg/l)

Leptin level (μg/l)	Total mortality	Cardiovascular mortality
2.6-5.4	1.07 (0.73-1.55)	0.79 (0.43-1.46)
5.4-9.8	1.13 (0.76-1.66)	1.14 (0.63-2.05)
9.8-18.7	1.15 (0.71-1.87)	1.37 (0.66-2.87)
18.7-184	1.17 (0.70-1.95)	1.25 (0.55-2.86)

High levels of leptin were not associated with mortality risk. More extreme cut-off points (10% of the highest levels) did not show increased risk either. Adjusting for other cardiovascular risk factors, correlates of leptin (body mass index, lipid level, post load glucose, fasting insulin) did not change the estimates. The associations did not differ between diabetic and non-diabetic subjects.

Conclusions - Despite its cross-sectional relationship with cardiovascular disease risk factors, leptin is not associated with mortality risk.

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PREVALENCE OF UNDIAGNOSED DIABETES AND CARDIOVASCULAR RISK FACTORS IN TONGA

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Introduction: Tonga is a developing Pacific nation with a population of 97,784 (59,526 over the age of 15 years). The health status of Tongans is increasingly threatened by the alarming growth of non-communicable diseases. A high and growing prevalence of complications has been documented in people with known diabetes but little was known about diabetes prevalence or risk factors for macrovascular complications in the undiagnosed population. **Aim:** To determine the prevalence of undiagnosed diabetes and cardiovascular risk factors in the Tongan population. **Methods:** Over 600 people (>15 years old) not known to have diabetes were randomly sampled from the general community to reflect the population as per the 1996 census. They were assessed for BMI, WHR, BP, fasting BGL, HBA_{1c} , lipid levels, microalbuminuria, % body fat, ECG, family history of diabetes, personal cardiac history. Details on food intake, smoking and physical activity were collected by questionnaire administered by a trained health officer. An oral glucose tolerance test was performed where the fasting blood glucose was >5.0mmol/L and <11.0mmol/L or if HBA_{1c} was elevated. Samples were transported and analysed in Australia. Statistical analysis was performed using SPSS for windows v6.1. **Results:** From 608 survey participants, it was determined that the overall age adjusted prevalence of undiagnosed diabetes was 13.4% (95%CI 10.0-16.8), for males 11.7% (95%CI 7.0-16.3) and females 14.8% (95%CI 9.9-19.7). The overall age adjusted prevalence of IGT in the Tongan population was 6.9% (95%CI 6.4-12.3), for males 10.4% (95%CI 6.0-14.8) and females 8.6% (95%CI 4.5-12.6). For people aged >45 years, the prevalence of diabetes was 18.1% and 11.8% for IGT. The mean BMI was 31.7, 55.6 % of people were obese and 29.9% were overweight The mean WHR was 0.86. A lying systolic blood pressure of >140mmHg was recorded for 24.8% and a lying diastolic blood pressure of >90mmHg in 16.1%. Abnormal results for cholesterol (32.95%), triglycerides (20.7%) and creatinine (2.3%) were also recorded. **Conclusions:** These findings represent a very high prevalence of undiagnosed diabetes and IGT. This information will support targetted health planning, delivery and evaluation.

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The DiaCard Study: European Diabetes Epidemiology Survey of Diabetes, Cardiovascular disease and the impact of cardiovascular risk factors

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Aims: Development of a risk score model for cardiovascular morbidity, mortality and effect of intervention in diabetes.

Materials: The DECODE Study: 25 European occupational or population based studies, all with OGTT. Estimated number of individuals with diabetes is about 3500

Methods: The impact of cardiovascular risk factors on cardiovascular morbidity and mortality in diabetes and impaired glucose tolerance, will be estimated from centres participating in the DECODE study. Data will be harmonised and pooled. Based on the survival analysis a risk score for individuals with diabetes will be made, making it possible to estimate the 1 and 5 year risk of death, acute myocardial infarction and stroke. The effect of risk factor intervention on cardiovascular disease will be estimated based on randomised controlled clinical trials for dyslipidemia, hypertension and metabolic regulation.

The risk score and estimated effect of risk factor intervention will be expressed as algorithms for use in a computer-based DiaCard Programme, based on the model of the PreCard Programme.

Prospects: The programme is designed for physicians treating diabetes and will enable the physician to calculate the absolute risk of the diabetic patient based on the patients risk profile and the estimated effect of risk factor modification. This information will be guided by suggestions for individual treatment goals based on current guidelines. The goal is an improved intervention strategy.

The DiaCard Programme risk score will be validated and tested as part of the prevention part of the large population based prevention studies Inter99 and DiaRisk in Copenhagen County.

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PREVALENCE OF TYPE 2 DIABETES AND OTHER METABOLIC SYNDROME COMPONENTS IN NORTHERN INDIGENOUS POPULATION OF SIBERA

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The objective of this study, a population based survey, was to determine the prevalence rate of type 2 diabetes, obesity, hypertension, dislipidaemia, insulin resistance among small ethnic group of northern aborigines living in a large Siberian territory named Evenkia. The Evenks still continue to follow their native traditions of lifestyle and nutrition. Reindeer-breeding and hunting are the main aspects of their activity. A total of 596 Evenks aged 18+ years in 5 communities in the Baikit district of Evenkia (89.9% eligible participants) were screening. The prevalence of overweight (BMI ≥25 kg/m^2) in this population was 31,9% (95%CI: 27.9-35.8), hypertension (BP ≥160/95 mmHg) was 16,6 % (13.6-19.7). Blood triglycerides >2.2 mmol/l was present in 3.5% (2.2-5.2), total cholesterol >6.5 mmol/l had 10,9% and HDL-cholesterol <0.9 mmol/l was present in 5.2% (1.9-8.4) of indigenous people. No cases of glucose intolerance were found in this study. Twenty-eight percent of aborigines had a combination of two or more symptoms of metabolic syndrome. Mean (±SEM) basal insulin levels were 43.1±2.1 and 47.1±1.8 pmol/l for men and women respectively and only one subject (woman) had insulin levels below 160 pmol/l. Body mass gain in Evenks did not affect to lipid metabolism evidently. The prevalence rate of high levels of triglycerides in overweight and in normal subjects were 3.5% and 2.5%. Low HDL-cholesterol had 5.3% and 5.4%, higher total cholesterol had 13.7% (6.7-20.4) and 9.1% (3.12-15.8) of overweight and normal subjects respectively. There were no differences in C-peptide blood levels in these groups. However the hypertension prevalence in overweight group was higher (25.8%, CI: 19.1-32.8) than in normal group (7.1%, 4.2-10.8, $p\chi^2<0.001$). Our data indicates that diabetes among Evenks is still rare. The total prevalence of type 2 diagnosed diabetes in all northern aborigines live in Taimir peninsula and Evenkia territory is 0.18% (0.9-2.8). Other components of metabolic syndrome in northern Siberian indigenous population are not common. Overweight in indigenous people is less accompanied by insulin resistance.

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INCREASED BONE MASS IN MIDDLE-AGED WOMEN WITH IMPAIRED GLUCOSE TOLERANCE.

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Aims: The aim of the study was to investigate the prevalence of impaired glucose tolerance (IGT) and if it was associated with altered bone mass in a geographically defined population of middle-aged women living in southern Sweden. **Materials and Methods:** The population comprised 5000 subjects who underwent medical and psychosocial examinations. The results are based on analysis of questionnaires and laboratory screening of biological variables. A 75 g oral glucose tolerance test (OGTT) was performed on women with metabolic disorders (n=2070) and wrist bone density was measured on all 5000 women by means of dual energy X-ray absorptiometry, and expressed as bone mineral density (BMD) ($g\ m^{-2}$) and further as standard deviation (SD) from age matched controls (Z-score) as well as from young healthy women (T-score). Data on numbers and means (SD) are presented and Wilcoxon´s rank sum test and chi-squared test were used. **Results:** The results show, that out of the 5000 examined women, 610 (12.2%) were glucose intolerant; 95 (1.9%) women had IFG, 399 (8.0%) IGT and 116 (2.3%) were diagnosed with diabetes. Adding the 76 (1.5%) women who had a diagnosis of diabetes prior to the study makes a total of 192 (3.8%) women with diabetes and all together were 686 (13.7%) glucose intolerant. The bone mass was higher in subjects using antihypertensive drugs ($p < 0.01$) and in those with a diagnosis of diabetes prior to the study ($p < 0.01$) as well as in those with a family history of diabetes ($p < 0.05$). Subjects with IGT had higher BMD (p=0.027), Z-score (p=0.0007) and T-score (p=0.0001) than normal subjects. This was even more pronounced than in diabetic subjects ($p < 0.01$ for Z-score and T-score but BMD non-significant). **Conclusions:** This study shows that metabolic disorders connected with diabetes in middle-aged women are associated with increased bone mass and that this seems to be most evident among subjects with IGT.

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Detection of Type 2 Diabetes According to WHO and ADA Diagnostic Criteria in Polish Population (Screen-Pol Study).

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An association between the complication of diabetes and hyperglycaemia has been documented in diabetes type 2. Early diagnosis and treatment of diabetes may reduce the risk of developing the complications. The new ADA diagnostic criteria for epidemiological purposes recommended a fasting venous plasma glucose concentration (FPG) instead of oral glucose tolerance test (OGTT). However, some of the studies have demonstrated a disagreement between the two criteria. The aim of study was comparison the prevalence of carbohydrate disturbances according to WHO and ADA diagnostic criteria. 285 general practitioners (GP) situated in whole country were screened 100 consecutive patients > 45 years. 27065 subjects were included to study. 3271 (12,1%) have previously diagnosed diabetes. All rest 22704 subjects (group W1) have determined a random capillary blood glucose concentration. 3420 of them (group W2) have glucose level >100 mg/dl and in this group FPG was determined. In 987 subjects (group W3) with FPG > 110 mg/dl he OGTT was performed. WHO and ADA criteria were used to diagnose abnormal glucose tolerance. The mean random glucose concentration in groups W1, W2 and W3 were 90,4+0,13; 129,4+0,39 and 149,9+1,45 mg/dl (p.>0.01), respectively. FPG were in group W2: 95,1+0,30 and in group W3: 132+0,93 mg/dl (p.>0.01). The number of subjects (and % of total tested population without of previously diagnosed diabetes) identified by each criteria is presented below:

	WHO	ADA
Impaired fasting glucose (IFG) or	-	454 (1,91%)
Impaired glucose tolerance (IGT)	265 (1,11%)	-
Diabetes	282 (1,19%)	272 (1,14%)
Unsolved between IFG/IGT or DM	160 (0,67%)	170 (0,71%)

In conclusions: Screening of diabetes by GP doctors is feasible and simple procedure. The rate of glucose undiagnosed disturbances according to WHO and ADA diagnostic criteria were 2,97% and 3,76%, respectively. Over 30% disagreement between the WHO and ADA criteria was observed. Total rate of diagnosed and unknown diabetes in tested population was 13,3%.

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HIGH PREVALENCE OF UNDETECTED DIABETES MELLITUS IN RURAL POPULATION IN EASTERN POLAND.

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Almost half of the inhabitants of Lublin Region /Eastern Poland/ and 1/3 of the whole population of Poland is a rural population. It is a social group which works only on own farms and that's why it is not a subject of periodical medical examinations. **The aim** of the study was to asses the prevalence of diagnosed and undetected diabetes mellitus in villagers aged 35 and more and to compare it with prevalence in urban population of our region. **Materials and Methods:** A two layer draw was applied: two groups of 3000 people were drawn from two samples, one from the Lublin town and the other one from the rural areas, each comprising 100 000 inhabitants. So far 1754 people have been examined: 1029 from Lublin and 725 from the country. Blood glucose (with Accu–Trend glucometer) and serum insulin (RIA) - fasting and 2 hours after an oral load of 75g of glucose were measured. The responsiveness rate in the rural areas was 60% and 48% in the town. **Results:** We found diabetes in 9,3% of the rural population /known – 4,4% and undetected – 4,9%/ and in 7,5% of the urban population /known - 5,0% and undetected - 2,5%/. 13,5% of the examined had positive family history of diabetes. Obesity (BMI>30kg/m^2) was diagnosed in 24,0% of women from the town and in as many as 43,5% of women from the rural areas, while in men it was found in 24,2% and 24,7% respectively. In the obese people diabetes was found in 15,6% of the cases and in as many as 46% was newly diagnosed. **Conclusions:** In the Lublin Region the population is about 1 million, what means that there is not less then 37 500 undiagnosed diabetics.

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REPRODUCIBILITY OF DIAGNOSTIC TESTS FOR DIABETES AND MORTALITY IN THE HOORN STUDY

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Aims - In epidemiological studies, the diagnosis of diabetes (DM) is often based on one abnormal diagnostic test (fasting or 2-hour glucose). In clinical practice however, the diagnosis should be confirmed by repeated testing on another day. In the present study we analysed reproducibility of diagnostic tests at 2 occasions. In addition, we assessed mortality in accord with the diagnosis based on single or duplicate tests.

Materials and Methods - In this population-based cohort of 1094 elderly subjects without known diabetes, fasting (FPG) and 2-hour plasma glucose values after an oral glucose load (2hPG) were determined at baseline and after 2 to 6 weeks. Subjects were categorised according to both WHO- and ADA-criteria at both tests. During the follow-up of 9 years, 147 subjects (13.4%) died.

Results - For the duplicate tests kappas of 0.57 (95% CI 0.53 - 0.62) and 0.59 (95% CI 0.53 - 0.64) were found when using WHO-criteria and ADA-criteria, respectively. Using WHO-criteria, 56 subjects had only one abnormal glucose value, while 70 subjects had abnormal values at both tests.

Using ADA-criteria, these numbers were 46 and 59 respectively.

Table 1. Mortality in accord with diagnosis based on single or duplicate tests and for WHO- and ADA-criteria [N(% deaths)]

Criteria (mmol/l)	no diabetes	one test DM	both tests DM
WHO (FPG ≥ 7.8 or 2hPG ≥ 1.1)	968 (13.0)	56 (16.1)	70 (17.1)
ADA (FPG ≥ 7.0)	989 (13.1)	46 (13.0)	59 (18.6)

Conclusions - These results indicate that there is a fair to good agreement between duplicate tests after 2 to 6 weeks. Subjects diagnosed by WHO-criteria as having diabetes at only one of two tests appear to have already a higher mortality risk. This was not observed for the subjects diagnosed only once by the ADA-criteria.

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LOW SENSITIVITY OF THE AMERICAN DIABETES ASSOCIATION CRITERIA FOR DIAGNOSING DIABETES AND GLUCOSE INTOLERANCE

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Background. The usefulness and reliability of the new criteria for diagnosis and classification of diabetes proposed by the American Diabetes Association (ADA) have recently been shown to be disputable. **Aims.** To assess the sensitivity and specificity of the ADA criteria in Polish population using World Health Organisation (WHO) 75 g oral glucose tolerance test (OGTT) as gold standard. **Materials and methods.** The results of 1554 OGTTs performed consecutively in 1990-98 were available for analysis. Cases with fasting plasma glucose >140 mg/dl, any disorder likely to affect the test result or incomplete results were not included into the study. OGTT was performed according to WHO protocol at the university laboratory by the same staff throughout the whole analysed period. Plasma glucose was measured with glucose-oxidase method. The diagnoses of diabetes (DM), impaired glucose tolerance (IGT), and impaired fasting glucose (IFG) according to both sets of criteria were compared. **Results.** 1360 OGTT results fulfilled the criteria to be included into the analysis. The concordance between the ADA and WHO criteria is shown in the table; data are given as percentage of the population.

		ADA criteria			
diagnosis		DM	IFG	normal	prevalence by WHO
WHO criteria	DM	2.9	4.4	8.8	16.2
	IGT	1.9	3.7	19.6	25.1
	normal	0.4	3.2	55.0	58.7
prevalence by ADA		5.3	11.3	83.4	100.0

The sensitivity of the ADA criteria for diagnosis of diabetes was only 18.2% with 97.2% specificity, i.e. 81.8% of cases of diabetes were missed to diagnosis. 77.8% of glucose-intolerant subjects were considered normal according to the ADA criteria. The poor agreement between IGT and IFG was noted; only 14.6% of IGT subjects had IFG, and 32.5% of cases with IFG had IGT and 38.9% had diabetes. On the whole, the ADA criteria detected glucose abnormalities in 16.6% of the population, while the WHO criteria showed their prevalence to be 41.3%. **Conclusions.** New criteria proposed by the ADA have essentially low sensitivity for diagnosing diabetes when compared with OGTT-based WHO criteria, and may not be completely useful for glucose intolerance assessment in the studied population.

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COMPARISON OF THE 1985 WORLD HEALTH ORGANISATION AND 1997 AMERICAN DIABETES ASSOCIATION CRITERIA FOR THE DIAGNOSIS OF DIABETES MELLITUS IN SOUTH AFRICAN INDIANS AND AFRICANS IN DURBAN. RESULTS FROM TWO POPULATION-BASED STUDIES.

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Aims: The aim of the study was to compare the prevalence of abnormal glucose tolerance based on 1985 World Health Organisation (WHO) and American Diabetes Association (ADA) criteria, in two cross-sectional population-based studies in South African Indians (n:2479) and Africans (n:485) aged >15 yr.

Materials and Methods: A 75g OGTT was performed on all subjects. Both sets of diagnostic criteria were applied to the OGTT results. Based on WHO criteria, a subject was classified as Normal glucose tolerance (NGT), impaired glucose tolerance (IGT) or diabetes mellitus (D); using ADA criteria, a subject was staged as normoglycaemia, impaired fasting glucose (IFG) or D.

Results: In the Indian survey, the crude overall prevalence of D was lower using ADA criteria (7.6%) than with WHO criteria (10.1%); 7.1% had D, using both sets of criteria. The prevalence of IGT was 5.7% and that of IFG 2.4%. When subjects with known D were excluded (n:2296), the prevalence of D was lower using ADA criteria (2.8%) than with WHO criteria (4.7%); 2.5% were classed D, using both sets of criteria. The prevalence of IGT was 5.1% and that of IFG, 1.8%. The overall agreement between the WHO and ADA criteria was "fair to good" (K 0.46; 95% CI 0.43-0.49). In the African survey, the crude overall prevalence of D was similar using ADA (4.9%) and WHO (4.3%) criteria; 4.1% had D, using both sets of criteria. The prevalence of IGT was 6.6% and that of IFG, 4.3%. When subjects with known D were excluded (n: 470), the prevalence of D was higher with ADA criteria (3.0%) than with WHO criteria (2.3%); 2.1% had D, using both sets. There was "fair to good" overall agreement between the two systems (K 0.44, 95% CI 0.37-0.51).

Conclusions: This analysis of population studies in different ethnic groups with high and moderate D prevalence using WHO criteria, highlights the variability in prevalence rates using ADA criteria.

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OGTT is better than fasting glucose to screen for abnormal glucose tolerance in women with a recent history of gestational diabetes.

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American Diabetes Association 1997 (ADA) diagnostic criteria, recommends the use of fasting plasma glucose FPG to study different stages between normality and diabetes (DM). Moreover, ADA advises to test for diabetes in women with a history of gestational diabetes mellitus (GDM). **Aim.** To compare the transcendence of the application of ADA criteria and the 1985 WHO criteria in women with a previous recent GDM, after pregnancy. **Subjects and methods .** A sample of 118 women, who had been diagnosed of GDM, aged 34.0 ± 4.6 years, was studied after gestation. The response to an oral glucose tolerance test OGTT were recorded when regular menses were recovered after delivery. According to WHO criteria subjects were classified depending on the 2h-glucose (G-2h) in: normal glucose tolerance NGT, impaired glucose tolerance IGT and DM. Based on the FPG they were classified as normal fasting glucose NFG, impaired fasting glucose IFG (≥6.1mM <7 mM) and DM (<7mM). The kappa-statistic measure of agreement was used to compare the two set of diagnostic categories. A receiver operating characteristic (ROC) curve was constructed to study the relationship between sensitivity and specificity of the FPG to detect abnormal glucose tolerance (IGT+DM). **Results.** Applying WHO criteria: NGT=87%, IGT=11%, DM=2%. Instead, using ADA criteria we obtained: NFG=95%, IFG=2.5%, DM= 2.5%. The kappa-statistic measure of agreement (scaled from 0 to 1) was 0.34, p=0.000. It is a fair agreement in the Landis and Koch scale. The area under the ROC curve was 0.494 (a test not better than chance). **Conclusions.** FPG seems not an appropriate screening test in order to identify abnormal glucose tolerance in women with a recent history of GDM. In our opinion OGTT should be recommended for such a purpose.

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ADA CRITERIA DIFFERENTIATES BETTER THAN WHO CRITERIA INSULIN RESISTANCE SYNDROME IN SIBLINGS OF NIDDM PATIENTS

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Aims: To find a diagnostic criteria able to differentiate both glucose tolerance and other metabolic features associated with insulin resistance in siblings of patients with NIDDM. **Materials and Methods:** 158 non diabetic siblings underwent a standard OGTT (75 gr) and the results were analysed according to WHO (1985) and ADA (1997) criteria. **Results:** Results are shown in the table

		WHO			ADA	
	NIDDM	**igt**	**ngt**	**type2**	**ifg**	**nfg**
N.	23	44	91	35	32	91
Age (yrs)	56±2	55±1	50±1 *#	56±1	57±1	50±1 **ab**
Weight (kg)	76±3	74±2	70±1	78±2	70±2**a**	70±1 **a**
Glucose (mg/dl)	149±9	116±3 *	102±2 *#	150±5	117±1 **a**	97±1 **ab**
Insulin (µU/ml)	13±1	13±2	9±1 *#	16±3	12±2	8±1 **ab**
HOMA	4.6±0.5	3.9±0.7	2.3±0.2 *#	5.7±0.9	3.3±0.4 **a**	2.0±0.1 **ab**
HbA1c (%)	6.2±0.2	5.9±0.2	5.4±0.1 *#	6.2±0.2	5.6±0.1 **a**	5.5±0.1 **a**
Triglyc. (mg/dl)	167±16	171±22	111±6	186±25	144±13	113±6 **ab**
Cholest. (mg/dl)	228±12	224±7	218±5	242±9	240±9	206 ±5 **ab**

igt: impaired glucose tolerance; ngt: normal glucose tolerance
ifg: impaired fasting glucose; nfg: normal fasting glucose
(*)=p<0.05 vs niddm; (#)=p<0.05 vs igt (**a**)=p<0.05 vs type 2;(**b**)=p<0.05 vs ifg

Conclusions: ADA but not WHO criteria can differentiate both glucose tolerance and other metabolic features of Insulin Resistance Syndrome in siblings of patients with NIDDM.

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CARDIOVASCULAR RISK IN IMPAIRED GLUCOSE TOLERANT INDIAN POPULATION CONSIDERING WHO AND ADA CRITERIA

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In a household selected population of Asian Indian migrants of Guadeloupe, at high risk of diabetes and cardiovascular disease (CVD), the selected subjects presenting an impaired glucose tolerance were different according to either ADA or WHO criteria, defining 2 groups. **Aims :** to assess the cardiovascular risk factors (CVRF) in, the 2 groups compared to normals. **Material and methods :** The CVRF were defined as high BMI, WHR, blood pressure ≥160 /90mmHg, cholesterol, HDL chol, triglycerides (TG). Concordance for glucose tolerance was made by k concordance rate. The CVRF were compared in 3 groups, the IFG (ADA) classified subjects (G1), the IGT (WHO) classified subjects but considered as normals according to ADA (G2), and the normals classified as such by both ADA and WHO criteria (G3). **Results :** The 80 IGT subjects were divided in 60 normals, 19 IFG, 1 diabetic patient considering ADA criteria. The 67 IFG subjects were classified as normals (n=29) ,IGT (n=19) and diabetic (n=19) using the WHO criteria. The k concordance rate for the different glucose tolerance states was 0.44 (moderate concordance, $p<10^{-3}$). TG and HDL cholesterol levels tended to increase cardiovascular risk in G2 subjects (p=0.06) compared to G3 subjects. CVRF such as WHR (0.92 ±0.01 vs 0.88 ±0.01; p<0.01) , TG level (1.8 ±0.18 vs 1.3 ±0.01; p=0.02) and cholesterol levels (2.2 ±0.05 vs 2.0 ±0.04; p=0.05) were higher in G1 subjects compared to G2. G3 subjects were younger (p<0.01) than both G2 and G1 patients (40.2±1.0 vs 46.0±1.9 and 48.7±1.3). **Conclusion :** IFG patients had higher CVRF than IGT patients, who themselves tended to have higher risk than normals. If normals are younger, IFG and IGT have the same age thus probably corresponded to 2 distinct populations at risk of CVD. IGT justifying to be identify, a 2 hour glycemia measurement after glucose load may remain interesting.

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CARDIOVASCULAR RISK RELATED TO *WHO/ADA* DIAGNOSTIC CATEGORIES ON HIGH RISK SPANISH POPULATION.

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Aim: To research into the impact of the new ADA diagnostic criteria as for cardiovascular prognosis on high risk Spanish population.

Material and Methods: Two cross-sectional studies involving 7 primary health care centers in Catalonia (Spain) were revised. Individuals aged>40 year-old with any major risk factor for diabetes were screened according to the WHO rules using a 75 g oral glucose tolerance test (OGTT) to measure fasting plasma glucose (FPG) and 2h plasma glucose. An assessment of the cardiovascular risk using Framingham Hearth Study reference based on sex, age, body mass index (BMI), smoking, glucose tolerance, blood pressure and lipid profile (tCOL, HDLc, LDLc, TGC) was carried out.

Results: Diagnoses of 970 individuals, 453 males (46.7%), mean age 59 year-old and BMI 30.6 $kg.m^{-2}$ were revised. Among the 459 diabetic subjects according to either the WHO (OGTT) or the ADA (FPG) criteria, 314 (68.4%) were classified as having diabetes according to both sets of criteria (WHO and ADA). The overlap between subjects diagnosed as normal was a 56.8% and suffered a dramatic downturn (20.7%) with respect to Impaired Glucose Tolerance vs. Impaired Fasting Glucose. According to the WHO categories the 10-years cardiovascular risk results were: 8% for individuals with normal glucose tolerance, 12% for Impaired Glucose Tolerance and 27% for diabetic subjects. The results using the ADA-97 criteria were: 8% (normal FPG), 12% (Impaired Fasting Glucose) and 29% (diabetes).

Conclusion: Despite a low statistical concordance between both sets of criteria, the study evidenced an excellent agreement as for identifying overall cardiovascular risk associated to the theoretical equivalent WHO and ADA categories.

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WHAT SHOULD BE THE FASTING PLASMA GLUCOSE LEVEL FOR DIAGNOSING DIABETES IN THE SOUTH ASIAN POPULATION?

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Aim - We evaluate the diagnostic criteria of diabetes proposed by American Diabetes Association (ADA) with that of WHO in a South Asian population. **Materials and Methods** - This study is based on retrospective data of 96,674 oral glucose tolerance tests (OGTT) done from 1978 to 1996. Of the total 96,674 subjects, 3,412 and 93,262 had OGTT with 50g-glucose (50gGTT) and 75g-glucose (75gGTT) load, respectively, before and after the recommendation of WHO (1980) criteria. For comparison, 2-h post-load plasma glucose (2hPG) level of ≥ 11.1 mmol/l was taken as a gold standard. **Results** - Based on the standard criteria, 85,062 were diagnosed as diabetic and 8,180 as non-diabetic subjects. The subjects with FPG ≥7.0, FPG ≥7.8 and 2hPG ≥11.1 mmol/l were 92.2, 86.2 and 92.4 %, respectively. The cut-point 2hPG ≥11.1 corresponded well with FPG ≥7.0 even after stratification of age. For non-diabetic subjects (2hPG <11.1), there was a marked disagreement (92% vs 21%) between IGT (2hPG, 7.8-11.0 mmol/l) and IFG (FPG, 6.1-6.9). However, the agreement between ADA (IFG and DM) and WHO (IGT and DM) classification was moderate (kappa (κ) = 0.46). Compared with 50gGTT, 75gGTTshowed higher prevalence of diabetes (86.7 vs 95.6%) in the lean subjects (<50kg). **Conclusion** - Overall, the data suggest that taking 2hPG 11.1 as a standard cut-point for diabetes, the FPG value inclines more toward 7.0 with 75g-OGTT and 7.8 with 50g-OGTT. A different diagnostic approach for the lean subjects (<50kg) either reduced glucose load (~50g) or increased cut-point (FPG ~7.8mmol/l), may be considered.

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STEEP INCREASE IN DIABETES PREVALENCE IF BOTH FASTING AND 2HR GLUCOSE VALUES ARE USED

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Aims: To determine the impact of using fasting glucose values alone, versus together with 2hr glucose values on diabetes prevalence as suggested by the new WHO diabetes classification. **Materials and Methods:** The prevalence of Type 2 diabetes (DM) was ascertained in the multi-ethnic (Indian, Chinese and African origin) population of Mauritius during a follow-up survey in late 1998 when 6,294 subjects were screened for diabetes with an oral glucose tolerance test (OGTT) with whole blood glucose (BG) measurement. 70% of participants were part of a survey in 1992 and the remaining 30% newly recruited. Because there was no statistical difference between new and cohort subjects, both groups were analysed together. Cut-off values of FBG ≥6.1 mmol/l and 2hrBG ≥10.0 mmol/l were used for diabetes classification. **Results:** Diabetes rates for those 30 years and over (age-standardised to the 1997 Mauritian population) are shown in the Table.

Criterion	Type 2 Diabetes DM Prevalence % (95% CI)		
	Male	Female	Total
FBG	18.9 (17.4 - 20.4)	19.8 (18.4 - 21.2)	19.3 (18.3 - 20.3)
2hrBG	19.6 (18.1 - 21.2)	21.1 (19.3 - 21.4)	20.3 (19.3 - 21.4)
Either	22.6 (20.9 - 24.2)	23.9 (22.4 - 25.4)	23.2 (22.1 - 24.3)

Using only the 2hr value in comparison to only the fasting for classifying diabetes results in marginally higher prevalence (n.s.). But if diabetes is classified if either one of the two tests is in the diabetic range then the prevalence increases by an average of 20%. **Conclusions:** Combining the fasting and 2h thresholds in an either/or fashion (as recommended by the WHO) had a much greater effect on prevalence estimates than did changing from using the 2h threshold alone to using the fasting threshold alone.

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COMPARISON OF DIAGNOSTIC CRITERIA FOR ABNORMAL GLUCOSE TOLERANCE IN AN OBESE POPULATION.

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Aims. To compare the new criteria for abnormal glucose tolerance using those criteria of the American Diabetic Association (ADA) with those of the World Health Organization (WHO). The revised criteria for ADA use a fasting plasma glucose concentration >= 7.0 mmol/l for diabetes and between 6.1 and 6.9 mmol/l for IFG. **Materials and Methods.** Data were collected from 145 obese Caucasian subjects referred to us for gastroplasty (BMI>30, 117 females and 28 males, age range 17-62). Fasting and 2 hour glucose concentrations were measured during a 75g oral glucose tolerance test. **Results.** With WHO criteria, 31 patients were diabetic and 37 had impaired glucose tolerance (IGT), while with ADA criteria only 24 were diabetic and 18 had impaired fasting glucose (IFG). Therefore the incidence of diabetes using the ADA criteria decreased by 22.4% and that of Impaired Glucose Tolerance (IGT), (if accepted as equivalent to IFG), by 51.4%. Of 31 subjects classified as diabetic by WHO criteria 8 were normal by ADA criteria. Of 37 subjects classified as IGT by WHO criteria 29 were normal by ADA criteria and 1 was diabetic. Kappa statistics for agreement between the 2 classifications for abnormal glucose tolerance was good for diabetes (0.84) but poor for all abnormal glucose tolerance (0.55). **Conclusions.** The use of a fasting rather than 2-hour plasma glucose will cause a decrease in the prevalence of abnormal glucose tolerance in an obese and morbidly obese population. The degree of disagreement between the two classifications was much larger for those with IGT than diabetes although both were cause for concern. If the progression of AGT through IGT to diabetes is associated with increased risk to health then the identification of obese individuals at risk arising from abnormal glucose tolerance in relation to these two criteria deserves a prospective trial.

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PATHOPHYSIOLOGY OF IFG AND IGT IN A JAPANESE COHORT

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Purpose: Based on WHO criteria, pathophysiology of IFG and IGT was analyzed through OGTT follow-up study. Methods: Subjects were 7,324 non-diabetics (4,114 normal, 2,802 IGT and 408 IFG) among OGTT examinees registered in 1965-1996 Study was made on the association between DM development rate, PG and IRI levels in OGTT. Mean age at registration was 54.0 years and mean follow-up period was 7.2 years. Results: (1) In examining DM development rate in IGT by OGTT 2-h PG, the rate increased with elevation of 2-h PG being 45.4 /1000 PY at 2-h PG of 140-149 mg/dl, 54.5 at 150-159 mg/dl, 67.8 at 160-169 mg/dl, 77.1 at 170-179 mg/dl, 101.2 at 180-189 mg/dl and 104.0 at 190-199 mg/dl. When IGT was divided into 2-h PG<170 mg/dl (IGT-1) and 2-h PG $\geqq$170 mg/dl (IGT-2), the rate was 56.3 and 112.0/1000 PY, respectively. DM development rate of IFG was 51.6/1000 PY, being similar to the rate of IGT-1. Also, the rate of IGT-1 within 1 year after registration was 19.6/1000 PY, with no difference from 19.9/1000 PY of IFG, but was significantly lower than 68.6 of IGT-2. The rate of normal cases was significantly lower, being 3.7/1000 PY. (2) In comparing 1-h PG of IFG and IGT-1, the rate of $\geqq$ 160 mg/dl was 74.3% in IFG, 77.5% in IGT-1 and 90.0% in IGT-2. The frequency of 1-h PG $\geqq$ 200 mg/dl was 36.3% in IFG, with no difference from 34.2% in IGT-1, but it was higher in IGT-2, being 59.2%. (3) 1-hPG was not different between IFG and IGT-1, but fasting and 1/2-h PG were higher in IFG, but 2-h PG and 3-h PG were higher in IGT-1. IRI response in OGTT showed no difference in fasting and 1/2-h IRI levels, but 1-h level was 60.7μu/ml in IFG, being higher than 51.3μu/ml in IGT-1, while 2-h value was 42.7μu/ml in IFG, being lower than 56.3μu/ml in IGT-1. Conclusion: IFG and IGT-1 did not differ in DM development rate . Higher IRI response in OGTT 1-h is followed by decreased 2-h PG, and OGTT will become IFG. On the other hand, lower IRI response in OGTT 1-h and delayed hyper-IRI response will lead to IGT-1. It is suggested that different IRI response will contribute to different OGTT results.

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Concordance between the 1997 fasting ADA and the WHO criteria for the diagnosis of diabetes and the impact of the WHO consultative document criteria

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The WHO is currently considering new diagnostic criteria for the diagnosis of diabetes. A WHO provisional report proposed lowering the diagnostic fasting plasma (blood) glucose value to ≥7 mmol/l (6.1 mmol/l). A new category of impaired fasting glucose (IFG) is proposed: FPG ≥6.1 to <7.0 mmol/l. In addition, gestational diabetes (GDM) now includes gestational impaired glucose tolerance as well as the previous GDM. **Aim:** Our aim was to compare the diagnostic sensitivity of the ADA and WHO criteria and examine the impact of the WHO consultative document criteria on misclassification of subjects by either criteria. **Method:** We reviewed data obtained from oral glucose tolerance tests (OGTT) on 2769 non-pregnant and 989 pregnant subjects referred with suspected type II or gestational diabetes respectively over a 3 year period where OGTT were done on capillary plasma samples at the Royal Preston Hospital, Preston, UK. **Results:** The rate of diabetes among the 2769 individuals on using a 2 hour plasma glucose ≥12.2 mmol/l cut off was 12.2%, FPG ≥7.8 mmol/l was 5.5%, FPG ≥7 mmol/l was 10.7%, FPG ≥7.8 mmol/l or 2 hour ≥12.2 mmol/l was 11.8%, and when using FPG ≥7 mmol/l or 2 hour ≥12.2 mmol/l was 14.3%. A FPG ≥7 mmol/l misclassified 33% of subjects as non-diabetic classified as diabetic by a 2 hour PG ≥12.2 mmol/l, and a 2 hour PG ≥12.2 mmol/l misclassified 36% diabetic subjects by FPG ≥7 mmol/l as non-diabetic. Such misclassification was abolished by using the criteria proposed in the recent WHO consultative document when using FPG ≥7 mmol/l or 2 hour PG ≥12.2. FPG ≥6.1 mmol/l identified 23 out of 49 subjects classified as GDM among 900 pregnant females by using 2 hour PG ≥8.9 mmol/l, misclassifying 53% of subjects as non-GDM. **Conclusion:** It is concluded that the proposed WHO criteria for the diagnosis of diabetes identifies the highest number of diabetic subjects and abolishes misclassification of individuals that results when using the ADA or the current WHO criteria alone. It should be adopted for routine clinical practice.

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INSULIN SECRETION DEFICIT IN PRE- AND EARLY TYPE 2 DIABETES

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Type 2 diabetes (DM) is characterized by a deficit in early phase insulin response and high insulin levels in the late phase after an OGTT. So far little is known at which level of fasting and postchallenge (pc) hyperglycemia anomalies of insulin secretion can be observed. **Aims:** We therefore analysed (1) early and late pc real insulin response by the given plasma glucose (PG) concentration (2) occurence of insulin deficit by quintiles of fasting and 2h pc glucose level. **Material and Methods:** In the Risk Factors in IGT for Atherosclerosis and Diabetes (RIAD) Study 785 subjects at risk for diabetes were tested by a standard 75g OGTT. Inclusion criteria: age 40-70 years, familial history of diabetes, and/or obesity or hyperlipoproteinemia. Exclusion criteria: known diabetes, treatment affecting glucose tolerance. PG, real insulin (ins) and proinsulin were determined 30', 60', 90' and 120' pc. Early insulin secretion was calculated as a ratio of ins30'-ins0' to pg30'-pg0' (Δins/Δpg). Late phase was calculated as a ratio of insulin area under the curve (AUC) 30-120' to PG AUC 30-120' pc (QAUC). **Results:** 459 subjects had a normal glucose tolerance (NGT), 207 IGT and 119 a newly diagnosed DM according to the new provisional WHO criteria. If calculated by the corresponding PG levels a deficit of early insulin secretion and even in the late phase secretion becomes obvious: early Δins/Δpg was 2.01×10^{-4} in NGT, 1.83×10^{-4} in IGT and 1.65×10^{-4} in DM ($p<0.05$ NGT vs. IGT, DM; IGT vs. DM) and late phase QAUC was 59.4, 60.8 and 48.0 ($p<0.05$ DM vs. NGT, IGT). Consistent with this finding we observed an increasing concentration and percentage of proinsulin in IGT and DM vs. NGT at 30' pc. By quintiles of fasting as well as 2h pc PG a continuoes decrease in early and late phase insulin secretion was observed that was significant vs. bottom quintile at a fasting PG level of 6.06-6.57 mmo/l and 2h pc PG of 7.77-9.33 mmol/l. In conclusion our data show a deficit in early and late phase of insulin secretion as early as at the cut-off limits for impaired fasting glucose and IGT when calculated by the level of corresponding PG.

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FAMILIALITY OF QUANTITATIVE TRAITS ASSOCIATED WITH THE METABOLIC SYNDROME IN NORMOGLYCAEMIC OFFSPRING OF ONE TYPE 2 DIABETIC PARENT

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The metabolic syndrome is characterised by a cluster of disorders, type 2 diabetes, obesity, dyslipidaemia and hypertension, which are both genetically and environmentally determined. The present study was undertaken in order to describe the familiality of risk factors for the metabolic syndrome in 224 Danish Caucasian offspring of 60 unrelated type 2 diabetic patients. All offspring and 32 non-diabetic spouses were examined in the fasting state and underwent a tolbutamide-modified frequently sampled intravenous glucose tolerance test (FSIGTT) with estimates of insulin sensitivity (Si), glucose effectiveness (Sg) and acute insulin response 0-8 min (AIR) calculated by minimal model analysis. Familiality (h^2) of the examined traits was estimated using variance components analysis with adjustment for different covariates.

Trait	adjustment	h^2 (±SE)
body mass index (BMI)	age, gender	0.49 (±0.15)
waist/hip ratio	age, gender	0.41 (±0.14)
fat mass	age, gender	0.45 (±0.24)
diastolic blood pressure	BMI, age, gender	0.04 (±0.23)
systolic blood pressure	BMI, age, gender	0.23 (±0.24)
fasting s-leptin	BMI, age, gender	0.28 (±0.13)
fasting s-triglyceride	BMI, age, gender	0.20 (±0.13)
fasting s-cholesterol	BMI, age, gender	0.32 (±0.14)
fasting s-HDL-cholesterol	BMI, age, gender	0.33 (±0.25)
fasting s-insulin	BMI, age, gender	0.37 (±0.22)
Si	BMI, age, gender	0.10 (±0.27)
Sg	BMI, age, gender	0.51 (±0.15)
AIR	BMI, age, gender	0.75 (±0.15)

In conclusion, there is a significant familial effect of several known risk factors for the metabolic syndrome in offspring of type 2 diabetic patients, in particularly AIR, Sg and BMI. The quantitative traits with the highest heritability will be examined in an ongoing linkage study for quantitative traits.

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FAMILIALITY OF QUANTITATIVE TRAITS DERIVED DURING AN ORAL GLUCOSE TOLERANCE TEST IN NORMOGLYCAEMIC RELATIVES OF ONE TYPE 2 DIABETIC PARENT

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Several genes are likely to be involved in the glucose homeostasis in the fasting state and after a glucose load. The present study was undertaken in order to describe the familiality of quantitative traits which could be obtained during an oral glucose tolerance test (OGTT). Sixty families containing 224 offspring and 32 spouses of type 2 diabetic parents were examined by a 75g OGTT. Plasma glucose, s-insulin, s-C-peptide levels were analysed at 18 time points (-30, -10, 0, 10, 20, 30, 40, 50, 60, 75, 90, 105, 120, 140, 160, 180, 210 and 240 min). p-GIP and p-GLP-1 were analysed at the same time points except for -30, 50, 75, 105, 140, 160 and 210 min. Familiality (h^2) of the examined traits was estimated as the ratio of the additive genetic variance to the total phenotypic variance using a variance component model and adjusting for body mass index, age and gender. Familiality of each trait was examined 1) in the fasting state, 2) as incremental area under the curve (AUC) from 0-120 min and from 0-240 min 3) and as the highest h^2 obtained for a single time point.

h^2(SE)	Glucose	Insulin	C-peptide	GIP	GLP-1
Fasting	0.71 (0.15)	0.35 (0.22)	0.56 (0.15)	0.67 (0.21)	0.91 (0.32)
AUC_{0-120}	0.39 (0.24)	0.19 (0.25)	0.54 (0.22)	0.28 (0.22)	0.75 (0.23)
AUC_{0-240}	0.40 (0.24)	0.21 (0.25)	0.54 (0.22)	0.24 (0.22)	0.77 (0.15)
Peak	0.56 (0.24)	0.46 (0.24)	0.47 (0.23)	0.46 (0.25)	0.57 (0.15)
	(140 min)	(10 min)	(160 min)	(90 min)	(90 min)

The data show a high level of familility of GLP-1 with a very high fasting level. For all the other measured traits except insulin, there is a high level of familiality for fasting values, whereas there is a modest familiality of the measured variables after a glucose load. There is no unique time point that gives a higher familiality for several traits, but most peaks are reached after 1½ hour. The highly familial quantitative traits based on p-GLP-1 will be used to search for related quantitative trait loci.

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SHARED FEATURES IN TYPE 2 DIABETIC SIBLINGS WITHIN FAMILIES IN THE DIABETES IN FAMILIES STUDY

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Type 2 diabetes is a heterogeneous disease, but the extent to which the specific phenotypes 'breed true' within families is not known. The Diabetes in Families Study (DIF) is a collection of 386 sibships of type 2 diabetic patients with at least two additional siblings irrespective of diabetic status. **Aim**: To determine familial associations of specific diabetic phenotypes in the DIF study. **Methods**: Data on 243 diabetic patients in 115 sibships were analysed, age: mean (SD) 65.0 (9.9) years, duration median (interquartile range): 9.0 (4.0-14.0) years, proportion on sulphonylurea alone / metformin alone / combination of tablets / insulin treatment : 25% / 7% / 15% / 34%. Intraclass correlation coefficients (ICC) were calculated for various phenotypic traits relating to type 2 diabetes, using analysis of variance and covariance where appropriate. HOMA beta-cell function (%B) was calculated from fasting glucose and C-peptide. HOMA insulin sensitivity (%S) was not calculated for patients on insulin. **Results**: Significant ICCs (i.e. familial association) were found for age (0.67, $p < 0.001$), but not age of onset, BMI (0.37, $p < 0.001$) and waist circumference (0.39, $p < 0.001$) both after controlling for sex, %B (0.29, $p = 0.001$), but not %S, and HDL-cholesterol (0.36, $p < 0.001$), but not other cholesterol fractions or triglycerides. There was no familial association for the sex of the affected sibling or for blood pressure. Though BMI and waist circumference sibship means were correlated (0.79, $p<0.001$), neither correlated with sibship means of %B or HDL-cholesterol, nor were these intercorrelated. **Conclusions**: The significant familial associations for measures of obesity, beta-cell function and HDL-cholesterol, indicate that some features of type 2 diabetes 'breed true'. This may be due to either common genetic or environmental backgrounds or both..The independent assortment of these features contribute to type 2 diabetic heterogeneity.

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HERITABILITY OF THE FASTING AND 120 MINUTES BLOOD GLUCOSE CONCENTRATIONS IN YOUNG TWINS - A POPULATION-BASED STUDY

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Aims: A previous study from The Danish Twin Register demonstrated a heritability of 0.26 for fasting plasma glucose and a heritability of 0.52 for 120 minutes plasma glucose in elderly twins (age 55-74). The aim of this study is to elucidate the relative importance of genetic and environmental factors for glucose tolerance in a young population.

Materials and methods: Study subjects were Danish monozygotic and dizygotic twin pairs at the age of 18-45 years recruited from The Danish Twin Register. Subjects underwent a standardised 75 g OGTT after an overnight fast. Capillary blood glucose concentrations were measured before oral glucose ingestion and 120 minutes later. Data were tested for normality and thereafter analyzed by means of interclass correlations and classical heritability tests.

Results: Blood glucose values (0 and 120 minutes) from a total of 276 pairs of twins, 133 monozygotic and 143 dizygotic pairs, were available.The interclass correlations for fasting blood glucose were 0.559 ($p<<0.001$) for monozygotic twins and 0.240 ($p=0.004$) for dizygotic twins. The estimated heritability for fasting blood glucose was 0.638. The interclass correlations for 120 minutes blood glucose were 0.437 ($p<<0.001$) for monozygotic and 0.334 ($p<<0.001$) for dizygotic twins. The estimated heritability for 120 minutes blood glucose was 0.206.

Conclusions: The present results show that the fasting glucose and the glucose tolerance is under both genetic and environmental control, also in a young population. The difference between the previous and the present results might suggest that the relative impact of genetic and environmental factors changes with advancing age, and that a possible genetic defect in insulin secretion (represented by the two hour OGTT glucose concentration) is phenotypical expressed only in late life.

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INCREASED CENTRAL ADIPOSITY AND PAI-1 IN SIBLINGS OF TYPE 2 DIABETIC SUBJECTS WITH ELEVATED FASTING PLASMA GLUCOSE

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Aims: Insulin resistance is associated with clustering of cardiovascular risk factors and the development of type 2 diabetes. The aim of this study was to investigate features of insulin resistance and cardiovascular risk in siblings of type 2 diabetic subjects. **Materials and Methods:** Thirteen sibling pairs (6 male:7 female) matched for age and gender but discordant for fasting plasma glucose (FPG); i.e. one sibling with increased fasting glucose (IFG): FPG 6.0 - 7.7 $mmol^{-1}$ and a normoglycaemic sibling (NGT), FPG<6.0$mmol^{-1}$ were evaluated for anthropometry, visceral adiposity (CT Scan), HOMA derived insulin resistance (%S), insulin, lipids, plasminogen activator inhibitor antigen (PAI-1) and blood pressure (BP). **Results:** IFG and NGT groups were mean age (SD) 56.8 (8.7) vs 55.8 (8.4) yrs and mean FPG 5.2(0.35) vs 6.1(0.7) $mmol^{-1}$, respectively. Siblings with IFG compared to NGT siblings had increased mean fasting insulin; 86(iqr, 55,135) vs 66 (iqr, 48-92) $pmolL^{-1}$, p=0.05, and reduced %S 61(iqr, 39,94) vs 79(iqr,57,109), p=0.04. IFG compared to NGT siblings were more centrally obese with a greater waist circumference (94 ± 10.8 vs 88 ± 13.7) cm, p=0.01, and greater visceral adiposity tissue area (167.4 ± 77.4 vs 136.8 ± 64.9) cm^2, p=0.02. Mean PAI-1 antigen was increased in the IFG siblings: 26.3 (iqr, 15.1,45.6) vs 11.1 (iqr, 2.1, 23.3) ng/ml, p=0.0002, but no differences were detected in lipids or BP. Insulin resistance (%S) was negatively correlated with visceral adiposity (Rs= -0.5, p=0.007) and PAI-1 levels (Rs = -0.5, p=0.006). **Conclusions:** Early and minor increases in fasting plasma glucose are associated with features of insulin resistance including increased PAI-1 concentrations that predate the development of type 2 diabetes. These changes may contribute to increased cardiovascular risk in early dysglycaemic states.

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PHYSICAL ACTIVITY IN NON-DIABETIC RELATIVES OF TYPE 2 DIABETIC PATIENTS

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Lifestyle and genetic factors contribute to the pathogenesis of type 2 diabetes mellitus. Non-diabetic first-degree relatives of type 2 diabetic patients are at increased risk of developing diabetes; this is thought to primarily reflect hereditary influences but shared lifestyle factors may be important in familial type 2 diabetes. **Aims:** to examine self-assessed physical activity in first-degree relatives of type 2 diabetic patients. **Materials and Methods:** non-diabetic relatives (n=152) and control subjects with no family history of diabetes (n=147), matched for age, sex and social class, completed a structured question on the self-assessment of physical activity (derived from the Danish Health Interview Survey). Subjects were classified on an ordinal scale and differences compared by Chi squared test. **Results:** the relatives were significantly less active than the control subjects (P<0.05); 34.2% *vs* 21.8% were classified as having light, 63.8% *vs* 76.9% moderate and 2.0% *vs* 1.4% heavy levels of activity. When comparing males alone there were no differences in activity levels. However there was a significant difference between the activity levels of the female relatives and control subjects (P<0.05); 42.3% *vs* 25.6% light, 56.5% *vs* 74.4% moderate and 1.2% *vs* 0% heavy. **Conclusion:** relatives of type 2 diabetic patients are less physically active than control subjects; this appears to be due primarily to lower levels of activity in the female relatives. Decreased physical activity may play an important additional role in the development of diabetes in "at risk" relatives of type 2 diabetic patients.

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SSCP ANALYSIS OF GLUT1 GENE IN MODY

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Aims: Although several MODY genes have been identified, the genetics of a percentage of MODY patients remains to be elucidated. Genes involved in beta-cell function are obvious candidate genes. There are increasing evidences for a central role played by the GLUT1 glucose transporter in beta-cell glucose transport. Glucose transport appears to be largely mediated by the low-Km transporters GLUT1 and/or GLUT3, with the GLUT2 playing a distinct role more directly related to exocytosis. Moreover, GLUT1 mRNa is largely predominant in the human beta-cell, when compared to GLUT2. For these reasons we have decided to search for the presence of sequence variants within the exonic sequence of the GLUT1 gene in MODY patients. **Materials and Methods**: The 10 exon and all the promoter region of the GLUT1 gene were amplified by PCR, and the fragments were electrophoresed in non-denaturing polyacrilamide or Mutation Detection Enhancement (MDE) gels. **Results:** Eight out of the 10 exons have been analysed. Sequence variants have been detected within exons 1, 2, 4, and 5. The sequence variants of exons 2, 4 and 5 corresponded to simple nucleotide substitutions (GCC (Ala^{15}), TGG (Cys^{133}), CCA (Pro^{196}), respectively) already described.

The variants in exon 1 were not detected in previous studies. Five out of 39 (12.8%) MODY patients had these SSCP variants, which are contained within nucleotides –34 and –259, in an area containing Sp1 binding sites and tetradecanoylphorbol responsive elements (TRE). Sequencing of this region is under way. **Conclusions:** The possible presence of a mutation within this region of the GLUT1 may help to define a new subclass of MODY patients.

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SCREENING FOR MUTATIONS IN THE HNF-1β GENE IN SCANDINAVIAN FAMILIES WITH EARLY-ONSET DIABETES AND DIABETIC NEPHROPATHY

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Rationale: Mutations in the hepatocyte nuclear factor-1β gene (*HNF-1β*) have been associated with MODY and non-diabetic renal disease. **Aims:** To investigate whether *HNF-1β* contributes to early-onset diabetes and/or diabetic nephropathy (DN) in Scandinavian populations. **Materials and Methods**: The HNF-1β gene was screened for mutations with SSCP using a ABI377 DNA sequencer in 143 diabetic patients, 115 of whom had onset of diabetes ≤40 years, whereas 28 of them had DN. Observed allele frequencies were also compared with 92 nondiabetic control subjects. **Results**: We identified 5 novel sequence variations and confirmed two intronic polymorphisms (IVS8+48insC and IVS8–22C→T). The novel variants included two amino acid substitutions (A241T in exon 3 and G492S in exon 7), two intronic variants (IVS6+26C→T, IVS8–66C→T) and one nucleotide substitution (C→G) 31 bp upstream from the start codon. The allele frequencies of these variants did not differ between diabetic and control subjects. The A241T substitution, located in the homeodomain region, was identified in one patient with end-stage DN but in no control subject. Ten diabetic G492S mutation carriers had an earlier age at onset than nine non diabetic relatives without matution (31±15 vs. 57±15 years; P=0.003). No difference was seen in urine albumine excretion rate (82±212 vs. 18 ±22 μg/min), fasting plasma glucose (FPG, 9.1±4.2 vs. 7.1±2.8 mmol/L) or fasting insulin concentrations (12±6 vs. 16±11 mU/L). In these families neither was there any difference in FPG (4.8±0.5 vs. 4.7±0.6 mmol/L) or 30 min insulin concentrations (42±39 vs. 39±30 mU/L) during OGTT between three nondiabetic carriers and ten non-carriers of variant G492S. **Conclusion:** Although variants in the *HNF-1β* gene are rare in patients with diabetes, they are associated with an earlier onset of the disease. Due to incomplete segregation the final elucidation of their pathogenic role in diabetes and DN requires functional studies.

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PREVALENCE OF THE MATURITY ONSET DIABETES OF THE YOUNG (MODY) IN THE PEDIATRIC POPULATION OF THE ITALY'S MARCHE REGION.

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Maturity Onset Diabetes of the Young (MODY) is a subtype of type 2 diabetes characterised by an autosomal dominant inheritance and an early age of onset. To date five genes have been identified on chromosomes 20q(MODY1), 7p(MODY2), 12q(MODY3), 13q(MODY4) and 17cen-q(MODY5). **Aim:** the prevalence of MODY2 and MODY3 gene mutations, the most common forms of European Caucasian subtypes of MODY, has been studied in the pediatric population of the Italy's Marche Region. **Materials and Methods:** out of four thousand children and adolescents hospitalized in our Pediatric Department, during the last three years, for clinical problems not related to metabolic or genetic disorders and no taking drugs interfering with glucose metabolism, 20 (0.5%) have been identified having a mild fasting hyperglycemia (glycemia >5.6<6.7 mmol/l) and a family history of type 2 diabetes. The molecular study of MODY2 and MODY3 genes has been performed in all these subjects and their families. **Results:** Five children (25%) carried a MODY2 gene mutation. Two families, carrying the same missense mutation (A173S), has been found to be distant related, while the others showed different missense mutations (I404S, H380D, R275C). Three children (15%) carried a MODY3 gene mutation. In two unrelated families we identified the G31D missense mutation and in the third family the R272H mutation. Twelve children were MODY2/3 negatives. Nine of these 12 had a typical MODY phenotype while three had a very high insulin response to the IVGTT (>90° pcl) and one of the parents with a late-onset insulin-dependent diabetes B-cell antibodies negative. This phenotype reminds the recent described MODY subtype (MODYx) probably associated to a primary insulin resistance and to an early insulin-requiring type 2 diabetes. **Conclusions:** these data showed that the study of MODY2 and MODY3 gene mutations can diagnose only the 40% of MODY phenotypes in the pediatric population of the Central Italy. Out of this 40% of children and adolescents 25% carried the MODY2 and the 15% the MODY3 genes mutations.

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FUNCTIONAL STUDIES OF VARIANTS OF THE HUMAN INSULIN PROMOTER FACTOR-1 (IPF-1) OCCURRING IN EARLY- AND LATE-ONSET TYPE 2 DIABETES

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Focus on transcription factors expressed in pancreatic β-cells as potential diabetes genes causing both early- and late-onset type 2 diabetes has intensified after the discovery of transcription factors: hepatocyte nuclear factor-4α, -1α, and -1β as being the cause of maturity onset diabetes of the young (MODY 1, 3 and 5) and the insulin promoter factor 1 (IPF-1) as a MODY 4 gene.

Aim: To examine the IPF-1 gene as a potential diabetes gene in patients with either early- or late-onset type 2 diabetes. **Materials and Methods:** We have performed mutational analysis by PCR-SSCP and heteroduplex analysis of the coding exons in IPF-1 on genomic DNA from 8 Danish diabetic patients with maturity onset diabetes of the young (MODY) who had been excluded as MODY 1 and MODY3 and from 200 Danish Caucasian patients with late-onset type 2 diabetes. **Results:** We found a novel A140T missense mutation in exon 2 of the IPF-1 in one of the MODY patients and a D76N variant in exon 1 in one of the patients with late-onset type 2 diabetes. Both groups also had the previously reported G ins/del at nt. -108 in exon 1. The biological function of these IPF-1 variants was tested as transactivators of the human insulin gene promoter.

Coexpressed together with the coactivators neuroD and pan (E47), wildtype IPF-1 or the mutants: A140T and D76N were compared for transactivation of the human insulin promoter driving a luciferase reporter in NIH 3T3 cells. Compared to wildtype IPF-1, the A140T had activity levels of 82% (48%-116%); and the D76N, 43% (35%-51%). **Conclusion:** Our data suggest that the D76N variant of IPF-1 have reduced biological activity whereas the A140T probably has normal activity. Hence, it is unlikely that the A140T variant is pathogenic in the described MODY proband whereas the D76N may be pathogenic in the patient with type 2 diabetes.

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Hepatocyte Nuclear Factor-6: Studies of Associations between Genetic Variability and Type 2 Diabetes or Estimates of Insulin Secretion

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Since the transcription factor hepatocyte nuclear factor (HNF)-6 is an upstream regulator of several genes involved in the pathogenesis of maturity onset diabetes of the young (MODY), we have tested the hypothesis that variability in the HNF-6 gene is associated with subsets of type 2 diabetes or estimates of insulin secretion. We cloned the coding region as well as the intron-exon boundaries of the two exons of the human HNF-6 gene. We examined genomic DNA form 6 MODY probands without mutations in the MODY1, MODY3 and MODY4 genes and genomic DNA from 54 patients with late-onset type 2 diabetes by combined SSCP-heteroduplex analysis followed by direct sequencing of identified variants. We found two silent variants (Pro94Pro, Gly287Gly) and one missense variant (Pro75Ala). In an association study the allelic frequency of the missense polymorphism Pro75Ala was 3.2% (95% confidence interval, 1.9-4.5) in 330 type 2 diabetic patients and 4.2% (2.4-6.0) in 238 age-matched glucose tolerant control subjects (P=NS). There were no differences between carriers (N=19) and noncarriers (N=219) of this polymorphism in glucose-induced serum insulin and C-peptide release during an oral glucose tolerance test in 238 middle-aged, glucose tolerant subjects. Moreover, in genotype-phenotype interaction studies of 226 glucose tolerant offspring of type 2 diabetic patients and of 367 young healthy subjects, the carriers (N=21 and N=15, respectively) of the polymorphism did not differ from non-carriers (N=205 and N=352, respectively) in acute (0-8 min) serum insulin or C-peptide responses during an intravenous glucose tolerance test. In conclusion, mutations in the coding region of the HNF-6 gene are not associated with type 2 diabetes or with significant changes in insulin secretion among the Caucasians examined.

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MOLECULAR GENETIC ANALYSES OF NORWEGIAN FAMILIES WITH MATURITY-ONSET DIABETES OF THE YOUNG

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Aims: Maturity-onset diabetes of the young (MODY) is characterized by autosomal dominant inheritance, onset usually before the age of 25, absence of ketosis, and no absolute insulin requirement. At present five genetic subtypes are known (MODY1-5), with each subtype having its own discrete phenotype. Our research focuses on the genetic epidemiology of MODY in Norway with the aim of confirming the clinical diagnosis by molecular genetic means, allowing appropriate treatment decision and genetic counselling. **Materials & Methods:** We have collected blood samples from about 30 Norwegian MODY families. Mutations were identified by PCR-based tests, or by screening families with microsatellite markers for known MODY genes, followed by sequencing of the candidate gene. In cases where only one affected member of a family was available for analysis, known MODY genes were sequenced directly. **Results:** We have so far identified five MODY3 families with the following mutations in the HNF-1α gene: P291fsinsC (two families), R171X, R229Q and R263C. In addition, one family had a V62A mutation in the glucokinase gene (MODY2) and one family carried an inactivated HNF-1β gene (MODY5). Since alterations of the HNF-1α gene, in particular the P291fsinsC mutation, seem to be a common cause of MODY in several populations, we developed a simple, PCR-based test for this so-called "hot-spot mutation" of MODY3. However, P291fsinsC was present in only two of 25 tested families. Haplotyping suggested that these two families are of a common origin. **Conclusions:** Genotyping of Norwegian families has shown the presence of MODY2, MODY3 and MODY5. The MODY3 "hot spot mutation" does not appear to be particularly common. Our patient material might be useful for identifying novel MODY genes.

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LOSS OF FUNCTION MUTATION IN THE HNF-1β GENE ASSOCIATED WITH RENAL DYSFUNCTION, DIABETES, GENITAL MALFORMATIONS

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Aims: Mutations in the hepatocyte nuclear factor (HNF)-1β are the cause of one form of maturity-onset diabetes of the young (MODY), MODY5. We have studied a Norwegian family (N5) ascertained through a female proband with frequent urinary infections, retarded motoric development, and a failure to thrive from birth on due to severe kidney malfunction. At the same time, this patient developed a type 2 diabetes mellitus that is presently being treated with insulin. We suspected MODY5 in family N5. **Materials and Methods:** In family N5, the co-segregation of renal dysfunction and diabetes, both of variable severity, occurs in five family members in three generations. We have screened the nine exons, flanking intron sequences, and the minimal promoter region of the HNF-1β gene for mutations and performed functional studies. **Results:** The sequence of the HNF-1β gene revealed a heterozygous 75 bp deletion in exon 2 (409-483del) resulting in an in-frame deletion of amino acids Arg137-to-Lys161 (R137-K161del). This deletion is located in the pseudo-POU region of the protein and co-segregated with kidney disease and diabetes implying that it is the cause of both disorders. Functional studies of R137-K161del HNF-1β revealed that it could not bind an HNF-1 target sequence or stimulate transcription of a reporter gene and indicated that this was a loss-of-function mutation. DNA binding studies by gel supershift assays in vitro and in vivo resulted in an inability of the mutant protein to bind to its binding side, therefore lacking the ability to transactivate downstream target genes. In addition, two of four female carriers with this mutation had vaginal aplasia and rudimentary uterus (Müllerian aplasia). **Conclusions:** Since the original report of Horikawa et al. another family with a HNF-1β mutation has been described. All the subjects in these three families (our family included) with HNF-1β mutations are characterized by early-onset and progressive renal dysfunction and/or diabetes mellitus of variable severity suggesting that subjects with these clinical features should be screened for mutations in HNF-1β. The identification of other families will reveal if HNF-1β mutations result in a distinct clinical syndrome characterized by renal dysfunction and diabetes mellitus.

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STUDIES OF A NOVEL MISSENSE MUTATION AT CODON 647 IN INSULIN RECEPTOR SUBSTRATE-2 USING THE YEAST TWO-HYBRID SYSTEM

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We have recently identified a novel heterozygous variant in the gene encoding human IRS-2 causing an amino acid substitution at codon 647, Leu647Val. The amino acid variant is located in the kinase regulatory loop binding (KRLB) domain, which is important for the interaction between IRS-2 and the insulin receptor (IR). The variant is less than 20 amino acids from two tyrosine residues which in mice have been demonstrated to contribute significantly to the interaction with the IR. Furthermore, the variant is positioned only 6 amino acids from Tyr 653 residing in a YMXM motif, which upon phosphorylation may interact with the SH2 domains of p85α, the regulatory subunit of PI3-kinase. In an association study, the variant was found in 3 of 413 diabetic patients and in none of 280 matched glucose tolerant control subjects. Due the potentially important location of the variant in IRS-2, we used the yeast two-hybrid system to examine whether the mutated IRS-2 had any impact on the interaction with the IR and p85α. Yeast cells were cotransformed with either wildtype or mutated murine IRS-2 KRLB domain and the IR cytoplasmic domain and p85α, respectively. The interaction between the proteins was estimated by measuring the β-galactosidase activity in transformed yeast cells. The interaction between the IR and the mutated IRS-2 KRLB domain showed no significant difference as compared to the interaction between IR and wildtype IRS-2 KRLB domain (relative activities (mean, 95% CI): wt 100% vs. Leu647Val 119% (84 - 154)). Similarly, there were no differences observed in the interaction between p85α and wildtype or mutated IRS-2 KRLB domains (wt 100% vs. Leu647Val 106% (101-112)). In conclusion: an amino acid substitution at codon 647 in IRS-2 has so far only been detected in type 2 diabetic patients. The variant showed no major impact on the interaction with neither IR nor p85α in the yeast two-hybrid system. However, the present study was constrained by the use of the IRS-2 KRLB domain instead of full length IRS-2. Therefore, further functional studies as well as segregation analyses in diabetic families are required to elucidate a potential diabetogenic role of this mutation.

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EVIDENCE FOR COMMON GENETIC CONTROL OF METABOLIC SYNDROME X AND TYPE-1 AS WELL AS TYPE-2 DIABETES

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Aim: Up to now there is a number of studies leading to genetic dissection of phenotypes such as hypertension, obesity or dyslipidemia, but there are no studies with animal models developing all these phenotypes known as metabolic Syndrome X. That was mainly attributed to the lack of a suitable animal model. This problem was overcome by establishment of WOKW rat as animal model for the metabolic Syndrome X manifesting moderate hypertension, severe dyslipidemia, obesity, impaired glucose tolerance and hyperinsulinemia. The aim of our study was the genetic dissection of syndrome X in the WOKW rat. **Materials and Methods**: WOKW rats (W-istar O-ttawa K-arlsburg RT1^u) were reciprocally crossed with DA rats to produce F1 hybrids, which were further intercrossed to generate F2 populations. 150 F2 male rats were used for quantitative trait locus (QTL) analysis by a genome-wide scan approach using 126 microsatellite markers. A linkage between phenotype (impaired glucose tolerance, dyslipidemia, body mass index, body weight, hyperinsulinemia) and genotype was determined using MAPMAKER/Exp and QTL computer program. **Results**: We found a major QTL for glucose metabolism on chromosome 3 at *D3Mit3* locus (lod score 5.0) and further QTLs influencing obesity and body weight on chromosome 1 between *D1Mit5* and *D1Mgh12* (lod score 4.1 and 4.9) and on chromosome 5 near *D5Mgh6* (4.5 and 4.2). Genetic determinants affecting triglyceridemia are mapped to chromosome 4 close to *D4Mgh2* (lod score 5.2) and affecting HDL-cholesterol to chromosome 17 near *D17Mit2* (lod score 4.4). In addition, hyperinsulinemia appears to be a result of interaction of loci on chromosomes 1 and 6, which were previously described as loci influencing diabetes development in the BB/OK. **Conclusion**: The genetic analysis of the Syndrome X in the WOKW rat manifesting a complex of disorders of the syndrome, clearly demonstrates not only its polygenic nature, but also indicates that the features of this syndrome might be at least partially under common genetic control. Furthermore, the fact, that certain regions found in our study are consistent with those previously suggested to be involved in type-1 and type-2 diabetes mellitus brings an evidence about close genetic relationships between syndrome X and diabetes mellitus.

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NOVEL AND SEX-DEPENDENT QTLS IN TRAITS OF THE METABOLIC SYNDROME X USING WILD RATS (RATTUS NORVEGICUS) AS DISEASE-RESISTANT COUNTERPART

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Aim: The spontaneously hypertensive rat (SHR) is thought to be an animal model for human metabolic Syndrome X including obesity, hypertension, dyslipidemia, glucose intolerance and insulin-resistance. To dissect the traits of such a complex disease, the SHR is normally crossed with disease-resistant rat strains. However, comparing the traits of the metabolic Syndrome X between so-called disease-resistant inbred rat strains (DA, F344, LEW, WKY, BN) and wild rats, we could clearly demonstrate that most, probably none, of our inbred rat strains is actually disease-resistant for metabolic as well as blood pressure disturbances. That prompted us to use wild rats for crossing studies to genetically dissect some features comparable with human metabolic Syndrome X. **Materials and Methods:** One male wild rat was terminatively crossed with SHR females. The (Wild x SHR) F1 hybrids were transferred into a pathogen free environment by wet-hysterectomy. One F1 female rat was backcrossed onto hypertensive SHR rats resulting 37 male (M) and 35 female (F) first backcross hybrids which were phenotypically characterised for body weight (BW), blood glucose (Glu), triglycerides (Tg), cholesterol (Chol) and systolic blood pressure (SBP) 3 times between 12 and 14 weeks of age. A genome-wide scan was carried out using 221 microsatellite markers on 20 autosomes and X chromosome. The MAPMAKER computer package was used to define quantitative trait loci (QTLs). **Results:** Significant linkage (lod score > 3.3) was found for BW on chromosomes 10 (M) and 18 (F), for Glu on chromosome 15 (M, F), for Tg on chromosome 17 (M, F) and for Chol on chromosome 14 (M, F). Suggestive linkage (lod score > 1.9) was determined for BW on chromosomes 3 (M) and 19 (F), Glu on chromosomes 17 (M) and 20 (F), for Tg on chromosome 6 (M, F) as well as SBP on chromosomes 2 and 7 (M) as well as 5 and 11 (F). **Conclusion:** In contrast to conventional crossing studies using inbred rat strains, this is the first study demonstrating novel and sex-dependent QTLs in traits of the metabolic Syndrome X. Wild rats as disease-resistant counterpart in crossing studies may help to identify essential major genes in animal models of complex human diseases in general.

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IMPACT OF THE XbaI POLYMORPHISM OF THE MUSCLE GLYCOGEN SYNTHASE GENE ON THE INSULIN RESISTANCE SYNDROME IN TWINS

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Aims: Insulin resistance represents a hall mark of various states of diseases predisposing to atherosclerosis including Type 2 diabetes mellitus, essential hypertension, obesity and dyslipoproteinaemia. A major defect in the insulin resistance syndrome is a defective activation of the muscle glycogen synthase (GS) by insulin. In some previous studies a polymorphism in intron 14 of the GS gene has been associated with Type 2 diabetes mellitus. The aim of this study was to elucidate the impact of this polymorphism on the insulin resistance syndrome and its various components in 283 pairs of monozygotic and dizygotic Danish twins. **Materials and Methods:** Plasma glucose and insulin were determined during 2 hour oral glucose tolerance tests. Fasting lipid levels and blood pressures were measured. In addition, body mass indeces and waist-to-hip ratios were calculated. The XbaI polymorphism was determined by PCR and enzymatic treatment with XbaI. All genotypic discordant twins were sequenced to confirm the discordance. **Results:** The frequencies of the non-cutting (A1) and cutting (A2) alleles were 0.95 and 0.05, respectively. The population consisted of 89,9 % A1A1 homozygotes and 10,1% A1A2 heterozygotes. No A2A2 homozygotes were detected. The population was in Hardy-Weinberg equilibrium. In the total twin population the A1A2 genotype had elevated diastolic blood pressure (mean 82 vs. 78 mmHg, $p<0,01$) and increased waist-to-hip ratio (mean 0,90 vs. 0,88, $p<0,05$) compared to the A1A1 genotype. The elevated diastolic blood pressure was confined to women with abnormal glucose tolerance (91 vs. 79 mmHg, $p<0,01$), whereas the increased waist-to-hip ratio was confined to men with abnormal glucose tolerance (0,99 vs. 0,96, $p<0,05$). Eleven genotypic discordant twin pairs were identified. In this unique subgroup the A1A2 heterozygotes were significantly more insulin resistant as assessed by homeostasis model assessment (HOMA) ($p<0,05$) compared to the A1A1 genotype. **Conclusions:** The intron14 XbaI polymorphism in the muscle glycogen synthase gene is associated with insulin resistance and components of the insulin resistance syndrome including elevated diastolic blood pressure and central obesity in Danish twins. The data showed that the association of the genotype and diastolic blood pressure or waist-to-hip ratio was influenced by gender.

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THE TUMOR NECROSIS FACTOR ALPHA –238 AND –308 PROMOTER POLYMORPHISMS ARE NOT ASSOCIATED TO INSULIN SENSITIVITY

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Aims: Tumor necrosis factor-α (TNF-α) is believed to influence skeletal muscle insulin resistance. Two G → A transitions in the promoter region of TNF-α at position –238 and –308 have been identified that could play a role in transcriptional regulation of the gene. Insulin resistance is an independent familial trait that predicts the development of type II diabetes. We wanted to investigate the influence on insulin sensitivity and insulin secretion of both polymorphisms in a well characterized population of young healthy relatives of patients with type 2 diabetes.
Materials and Methods: We examined 109 first degree relatives (FDR) of caucasian subjects with a history of type II diabetes who were younger than 50 years. Insulin sensitivity was determined by euglycemic-hyperinsulinemic glucose-clamp. The TNF-α –238 and –308 G → A promoter polymorphisms were determined by PCR and subsequent restriction enzyme analysis with *Msp* I.
Results: For the –238 polymorphism, 83 probands (76.1%) were homozygous for the G-allele, 25 probands (22.9%) were heterozygous, and 1 proband (0.9%) was homozygous for the A-allele. For the –308 polymorphism, 83 probands (76.1%) were homozygous for the G-allele, 24 probands (22.0%) were heterozygous, and 2 probands (1.18%) were homozygous for the A-allele. Probands with and without the polymorphism did not differ in insulin sensitivity ($p = 0.78$), insulin- and C-peptide concentrations in oral glucose tolerance tests ($p >> 0.05$).
Conclusions: We could not detect an association between insulin sensitivity or insulin secretion and TNF-α promoter polymorphisms in our collective of young healthy offspring of type II diabetes patients. The polymorphisms occur at the same frequencies in probands with either low or high insulin sensitivity.

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THE -258 G-A VARIANT OF THE GLUCOKINASE GENE HEPATIC PROMOTOR IS ASSOCIATED WITH HIGHER INSULIN SENSITIVITY IN CHINESE

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Aims: We investigated the effect of the -258 G-A variant of the hepatic-specific promoter of the glucokinase gene on plasma glucose and insulin levels in a Chinese population because in a previous study among 25 Afro-Americans with normal glucose tolerance (NGT), increased insulin resistance was found in 3 homozygous AA subjects. **Materials and Methods:** We studied 676 unrelated Chinese subjects (aged 50.3±12.5 years; mean ± SD) with NGT (n=351) or impaired glucose tolerance (IGT; n=325) according to WHO (1985) diagnostic criteria. Fasting and 2-hour plasma glucose and insulin levels were measured during a 75-g oral glucose tolerance test. The -258 G-A genotype was determined using PCR and AccI restriction fragment length polymorphism. **Results:** IGT subjects had significantly higher 2-hour insulin levels and 2-hour insulin/glucose ratios than those with NGT ($p<0.001$ and $p<0.01$ respectively), suggesting the presence of insulin resistance. The allele frequency of the A variant was 25% and 24% in IGT and NGT subjects respectively (P=NS). Among NGT subjects, no significant difference in fasting or 2-hour insulin or glucose levels was found for subjects with different genotypes. Among subjects with IGT, however, those with the A allele had lower 2-hour insulin levels [74.6(67.6-82.4) vs 90.2(81.8-99.3) pmol/mmol for GG, P=0.008] and 2h insulin/glucose [8.07(7.41-8.8) vs 10.2(9.24-11.2) pmol/mmol for GG, P=0.007] compared to subjects with GG. **Conclusion:** In contrast to the findings in Afro-Americans, these data suggest that the -258 hepatic glucokinase gene promoter A variant is associated with less insulin resistance in Chinese subjects with impaired glucose tolerance.

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PROMOTER VARIANT OF THE HEPATIC LIPASE GENE IS ASSOCIATED WITH INSULIN RESISTANCE

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Variants in the promoter of the hepatic lipase (HL) gene have been associated with components of the insulin resistance syndrome, e.g. high levels of triglycerides and low levels of high-density lipoprotein (HDL) cholesterol.
Aims: To investigate the association of the G-250A promoter variant of the HL gene with insulin-stimulated whole body glucose uptake (WBGU).
Materials and Methods: Genotypes of the G-250A polymorphism in the HL gene were determined in 110 randomly selected healthy subjects [82 males, 28 females, age 50.7±7.6 (mean±SD) years, body mass index 26.1±3.6 kg/m^2]. The rates of insulin stimulated WBGU were determined by the hyperinsulinemic euglycemic clamp in combination with indirect calorymetry.
Results: The A-250 allele of the HL gene was associated with low rates of insulin-stimulated nonoxidative glucose disposal (41.1±12.7 µmol/kg/min in 62 subjects with the G-250G genotype, 36.9±13.1 µmol/kg/min in 40 subjects with the G-250A genotype and 29.9±13.5 µmol/kg/min in 8 subjects with the A-250A genotype, p=0.012 adjusted for age and gender). No differences in the rates of insulin-stimulated WBGU and glucose oxidation were observed. In addition, the A-250 allele was associated with high levels of total triglycerides (p=0.009). No association between total or HDL-cholesterol and this polymorphism was observed.
Conclusions: The G-250A promoter variant of the HL gene is not only associated with dyslipidmias but also with a defect in insulin-stimulated nonoxidative glucose disposal. The mechanism by which this polymorphism could affect insulin sensitivity remains unknown.

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A Novel UCP3 regulatory region polymorphism is associated with body fat distribution in a South Indian Population

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Aims: Linkage between markers close to the uncoupling protein 2 and 3 genes (11q13) and resting metabolic rate and a pre-diabetic phenotype have been found. The syntenic region in mouse has been found to be linked to quantitative traits associated with obesity and diabetes. UCP3 expression in skeletal muscle has been found to be significantly reduced in Type 2 diabetics. UCP3 may therefore have an important role in body weight regulation and susceptibility to diabetes. By mutation screening of the UCP3 gene we have previously identified a novel point mutation (C to T) close to the TATAA box. Therefore the purpose of this study was to investigate a South Indian population for a correlation between this new variant and Type 2 diabetes and diabetes/obesity related traits . **Materials and Methods**: Two separate study groups were used, firstly families (n=83, 249 members) , comprising of a Type 2 diabetic proband and parents, and secondly a cross sectional survey (n=454). The variant was screened using a generated Hae III site PCR-RFLP digestion and gel electrophoresis. **Results**: In the South Indian families no association with Type 2 diabetes was found using the Extended Transmission Disequilibrium Test . Analysis of the parents found a positive association in the mothers for the presence of the T variant (p=0.006) and increased WHR (mean 0.92 vs 0.87 for presence of C allele only). No such association was found in the fathers (mean WHR 0.945 vs 0.953). In the cross sectional survey we were able to replicate the findings in the families, with a significant association in the females (n=220) between WHR and the UCP3 variant (p=0.039) ,but not in males (n=234)(p=0.6). **Conclusion**: No association was found with this UCP3 gene variant and Type 2 diabetes, however in Female South Indians the T allele was consistently related to increased central adiposity.

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STUDIES OF THE VARIABILITY OF THE GENES ENCODING IGF-I AND ITS RECEPTOR IN RELATION TO INTRAUTERINE GROWTH RETARDATION, INSULIN SENSITIVITY AND TYPE II DIABETES MELLITUS

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Human IGF-1 is an important regulator of many aspects of growth, differentiation and development and since a low birth weight has been associated with impaired glucose tolerance and type II diabetes in adult life we considered the genes encoding the IGF-1 and the IGF-1 receptor as candidate genes for intrauterine growth retardation, insulin resistance and type II diabetes. Here, we report the mutational analyses of the IGF-1 and IGF-1 receptor genes performed on genomic DNA from probands of 82 Danish type II diabetic families. All the coding regions and the intron/exon boundaries of the 5 exons of IGF-1 and the 21 exons of IGF-1R were analyzed for mutations using SSCP/heteroduplex formation analysis at two different experimental settings. No missense mutations were detected in the IGF-1 or the IGF-1 receptor genes. Two variants were identified in the IGF-1 gene; one intron variant and one silent variant at codon Arg21Arg, allelic frequency 0.02. Six silent variants and four intron variants were found in the IGF-1R gene. The six variants in the coding region and the allelic frequencies were: Ala211Ala, 0.01; Ser261Ser, 0.01; Gly271Gly, 0.02; Thr736Thr, 0.05; Glu1013Glu, 0.49; Tyr1316Tyr, 0.05. We evaluated the impact of the prevalent codon 1013 polymorphism in a population based sample of 380 young healthy subjects but no relationship between this variant and birth weight, birth length or insulin sensitivity were detected. Neither did we observe any significant differences in allelic frequencies of the codon 1013 variant between groups consisting of 246 type II diabetic patients (allelic frequency 0.47) and 415 matched glucose tolerant control subjects (allelic frequency 0.52). In conclusion, variability in the coding regions of the IGF-1 and the IGF-1 receptor does not associate with reduced birth weight, insulin sensitivity or type II diabetes in the Danish population.

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TYPE 2 DIABETES AND THE HUMAN HOMOLOG OF THE RAT INSULIN RESISTANCE GENE, *CD36*: NO LINKAGE OR ASSOCIATION IN EUROPEANS

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Aims: Defects in the *Cd36* gene lead to insulin resistance and defective fatty acid metabolism in the spontaneously hypertensive rat. This study aims to examine the role of variation in *CD36*, the human homolog, in the pathogenesis of type 2 diabetes. **Materials and Methods:** Nonparametric linkage was performed in 300 families (634 affected, 72 unaffected members) from the British Diabetic Association (BDA) Warren2 sibpair resource: each contains a type 2 diabetic sibpair of British/Irish origin (probands: median(range) age of diagnosis, 56(35-75)y; BMI 28.1(16.8-51.6)kgm^{-2}). Families were typed for the *CD36* region (chromosome 7) with ABI LMS2 markers, supplemented by an intragenic microsatellite (intron 3: LO6849), and analysed with GENEHUNTER v2. In addition, family-based association methods were used in 158 parent-offspring trios from the BDA Warren Trios repository, each ascertained via a Europid type 2 diabetic proband (age of diagnosis 40(25-58)y; BMI 30.8(19.7-59.6)kgm^{-2}), and typed for two common *CD36* variants of potential functional relevance, a 16bp deletion in exon 14, and a g→t variant at promoter position –53. **Results:** There was no evidence for excess allele-sharing in the sibpairs, with a NPL score at *CD36* of –0.74 (p=0.77) and exclusion of a gene with a sibling relative risk (λ_s)>1.5 from the 15cM interval D7S669-D7S657. In trios, there was no evidence for linkage disequilibrium between either variant and diabetes (exon 14, χ^2=0.59, p=0.44; promoter, χ^2=0.02, p=0.88; 2-locus TDT, p=0.33). Neither variant was associated with intermediate variables (age at diagnosis, weight, BMI, waist-hip ratio) in parents or offspring (all p>0.02, uncorrected). **Conclusions:** In European families, there is no evidence for linkage between *CD36* and type 2 diabetes nor of linkage disequilibrium with two common *CD36* variants. *CD36* is not a major gene for diabetes in this population, but minor effects for variants other than those studied cannot be excluded.

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GENETIC AND PATHOPHYSIOLOGICAL CHARACTERIZATION OF THE MAJOR DIABETES LOCUS IN GK RATS

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Genetic studies of the type 2 diabetes–like GK rat have revealed several susceptibility loci for the compound diabetes phenotype. Congenic strains for *Niddm1*, the major quantitative trait locus (QTL) determining postprandial glucose levels were established by transfer of GK alleles onto the genome of the normoglycemic F344 rat. Despite the polygenic nature of the diabetes in GK, the locus-specific diabetes-phenotype was retained in the congenic strain Niddm1a, containing a GK derived genomic fragment of 52 cM that includes the *Niddm1* locus. Furthermore, *Niddm1* was divided into two non-overlapping loci, physically separated in the two congenic strains Niddm1b and Niddm1i, with distinct metabolic phenotypes. Both strains displayed postprandial hyperglycemia and reduced insulin action in isolated adipose cells. Furthermore, Niddm1i exhibits a pronounced *in vivo* insulin secretion defect already at 65 days of age, while Niddm1b develops a relative insulin secretory defect at 95 days. This suggests that *Niddm1i* impairs mechanisms common to insulin secretion in pancreatic B–cells and insulin action in adipocytes. Niddm1b rats display increasing insulin resistance with age, resulting in obesity, hyperinsulinemia, and dyslipidemia. Moreover, the data indicate non-allelic interaction (epistasis) between *Niddm1b* and *Niddm1i* on postprandial glucose levels. This emphasizes the pathophysiological complexity of diabetes, even within an apparently single QTL, and demonstrates the potential of the GK model in dissecting disease pathways predisposing for type 2 diabetes and reducing complex phenotypes to bimodal mendelian traits suitable for positional cloning.

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Construction of Congenic Lines for the Locus *Nidd/gk1* Controlling Glucose Tolerance in the GK Rat.

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Genetic studies of the spontaneously diabetic (Type 2) Goto Kakizaki (GK) rat have demonstrated the importance of this model for the mapping of genes controlling diabetes phenotypes. Congenic lines from the GK rat are derived in order to test the effect of the *Nidd/gk1* locus on glucose tolerance and to refine its localisation on rat chromosome 1. Lines are derived through successive backcross breedings with non diabetic Brown-Norway (BN) rats, using a genetic marker assisted protocol to increase transfer efficiency of GK chromosomal intervals onto a BN genetic background. We have constructed two congenic lines for large segments of GK chromosome 1, together spanning over 95cM and containing the locus *Nidd/gk1* affecting glucose homeostasis: a line (1a) carrying 77cM of GK chromosome 1 (D1Wox18/D1Mgh12) and a line (1b) containing 20-26cM of GK chromosome 1 (D1Mit7/D1Mgh14). Intravenous glucose tolerance tests performed in 3 month-old animals show that glucose tolerance in rats from line 1b is not significantly deteriorated when compared to BN. In contrast, rats from line 1a show a significantly impaired glucose tolerance when compared to BN and line 1b rats. Cumulative glycaemia during the test is 4590±95mg/ml in BN, 4675±145 mg/ml in line 1b and 5260±103 mg/ml in line 1a, ($p<10^{-5}$ and $p<10^{-3}$ when compared to BN and 1b respectively). The effect on glucose tolerance was more pronouced in female than in male congenic 1a animals. These results confirm the existence of a QTL affecting glucose tolerance on chromosome 1 of the GK rat. Furthermore, preliminary phenotypic characterisation of these congenic lines suggests that a 20cM interval may be eliminated as a candidate region containing a gene substantially affecting glucose tolerance in this model. Further phenotpyic characterisation of sublines derived from congenic 1a will allow refinement of the position of susceptibility gene(s) at the locus *Nidd/gk1*.

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VARIATION IN THE GENE FOR THE SKELETAL MUSCLE UNCOUPLING PROTEIN (*UCP3*): A NOVEL PROMOTER VARIANT INFLUENCING FAT DISTRIBUTION.

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Aims: The *UCP3* gene maps to a region of chromosome 11 linked to metabolic rate in humans, and is the predominant UCP in skeletal muscle. Since dysfunction of this gene could reduce thermogenic capacity, thereby disturbing energy homeostasis and predisposing to obesity and type 2 diabetes, we have been cataloguing and evaluating genomic variation at this locus. **Materials and Methods:** For mutation screening, we assembled a panel of 30 women of mixed ethnic group, selected for a history of previous gestational diabetes (GDM) and high BMI (>28kgm^{-2}). All 8 exons and the core promoter were screened using both single-stranded conformational analysis and direct sequencing. **Results:** We have identified 3 variants of potential functional relevance: (a) G304A, producing Val→Ile at codon 102; (b) g→a in the splice donor site of exon 6; and (c) a c→t change in the promoter at position –55bp, which is 6bp upstream of the TATA box. These variants were assessed in larger cohorts including 74 women with previous GDM and 158 parent-offspring trios from the BDA-Warren Trios repository (ascertained through a GAD-antibody negative Europid proband with type 2 diabetes). The Val102Ile and exon 6 variants were only seen in AfroCaribbean subjects (both ~15% allele frequency). The promoter variant was present in all ethnic groups (frequency ~20%). In the Warren trios, we found no evidence for preferential transmission of either allele at this site (P=0.36). When family members were analysed for relevant intermediate traits, promoter variant genotype was associated with increased waist-hip ratio in parents (tt(n=4) 0.97(0.03); ct(n=134) 0.93(0.09); cc(n=185) 0.90(0.09), P=0.02[mean (SD)]). On further analysis, this relationship was confined to mothers (mothers P=0.007, fathers P=0.87). **Conclusions:** We have identified several variants in *UCP3*, some with ethnic-specific distributions. From data available, the c→t promoter variant is not a major susceptibility factor for type 2 diabetes, but it may influence fat distribution in later life.

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A NONSENSE MUTATION IN THE ISL-1 GENE IDENTIFIED IN A TYPE 2 DIABETIC PATIENT WITH STRONG FAMILY HISTORY.

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Aims: ISL-1 is one of the transcriptional factors with LIM homeodomain which is expressed in the islet cells. To find whether this gene was responsible for Japanese type 2 diabetes or not, we screened the ISL-1 gene for mutation. **Materials and Methods:** Seventy-seven Japanese Type 2 diabetic patients with family history and 180 non-diabetic subjects were screened by PCR/SSCP analysis. Statistical analysis was performed $\chi 2$ test Then, the effect of the mutation on ISL-1 function was examined by testing their ability to stimulate transcription of a human amylin promoter-linked luciferase reporter gene, using βTC3 cells. **Results:** A *C* to *G* change at 15 bp downstream from splice donor site of exon 3, a silent mutation (P168P) in exon 4 and a nonsense mutation (Q310X) in exon 5 were found. Allele frequencies of the two former variants were not significantly different between diabetic and non-diabetic subjects. (C to G change: diabetics 11 9% Vs non-diabetics 7.0%, P168P: Diabetics 30.7% Vs non-diabetics 34%, ns) The Q310X mutation, in the heterozygous state, was identified in a diabetic patient. This mutation located in the rear of homeodomain. It deletes 40 amino acids of the C terminal lesion. The 49 years-old patient with the Q310X mutation was diagnosed with Type 2 diabetes at 32 years old and has been treated with sulfonylureas. He had a history of insulin treatment for several weeks at age of 32 (onset time) and 42. His mother with diabetes and 14 years-old daughter with normal fasting glcose also carry the Q310X mutation. The Q310X exhibited a 50% reduction in activity compared to the wild-type. **Conclusions:** ISL-1 gene mutation is not a major susceptibility gene for Japanese common type 2 diabetes. But in this study, we found an interesting nonsense mutation in a type 2 diabetic patient with strong family history. This mutation decreased the transcriptional activity of ISL-1 in vitro pointing toward an important role of ISL-1 in the pathogenesis of Type 2 diabetes with strong family history.

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A NOVEL MICROSATELLITE MARKER LOCATED IN THE PROTEIN KINASE Cβ GENE: ASSOCIATION WITH DIABETES?

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Aims: Protein kinase Cβ (PKCβ) was associated with insulin resistance due to phosphorylation of the insulin receptor tyrosine kinase impairing its function. Insulin resistance plays an important role in the pathogenesis of type 2 diabetes (T2D). Therefore variants of PKCβ may be associated with the occurrence of diabetes. Recently, it was shown that microsatellites underlying selection pressure typically show multimodal distributions and may be excellent markers for gene clusters. We therefore searched for microsatellites with a non-Gaussian distribution in the PKCβ gene. **Materials and Methods:** Microsatellite markers were identified within the genomic sequence of PKCβ. For several markers primers were designed using "Oligo" software and their distribution was assessed. Marker genotypes were analysed manually by PCR amplification, electrophoresis on denaturing 5% polyacrylamide gels and silver staining. **Results:**One marker within the 3' region of the PKCβ gene consisted approximately of an $(AC)_{21}$-repeat and showed a bimodal distribution. The first peak comprised 5 different alleles and the second a single allele. Altogether, there were 9 different alleles resulting in a heterozygosity HET=0.85 and a polymorphism information content of PIC=0.83. The frequency of alleles in the first peak differed between T2D and controls: The frequency of allele "9" was 0.16 in controls (n=142 chromosomes) and 0.25 in T2D (94 chromosomes). By contrast the frequency of allele "7" was 0.23 in controls and 0.16 in T2D. The second peak did not differ between controls and T2D. **Conclusion:** A highly polymorphic microsatellite marker within the PKCβ gene is described. This marker shows overall a bimodal distribution. Allele frequencies within the first peak separate T2D from controls suggesting that this marker may be associated with T2D.

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Genetic Variability of the GLUT2 Promotor and Its Relationship to Type 2 Diabetes Mellitus

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Glucose transporter 2 (GLUT2) is together with glucokinase part of the glucose sensing machinery of the β-cell, and reduced GLUT2 expression or function could cause a dysregulation of glucose homeostasis and/or β-cell function. Previously, the coding sequence of the GLUT2 gene has been examined for genetic variants but so far no gene variants associated to type 2 diabetes have been identified. The aim of this study was to examine the promotor of GLUT2 for genetic variation and examine whether variants were associated with type 2 diabetes mellitus or measures of β-cell function. We have examined 1340 nucleotides upstream of the transcription initiation site in 36 type 2 diabetic patients by SSCP-heteroduplex analysis. Samples showing aberrant patterns of migration were sequenced directly. We have until now identified two nucleotide variants: a G to A substitution at position -447 and a T to C substitution at position -122 of the transcription initiation site. The G/A -447 variant was only identified in one type 2 diabetic proband and in her two children, but it was not detected among 363 other type 2 diabetic patients or among 245 glucose tolerant control subjects. The allelic frequency of the T/C -122 variant was 9.8% (95%CI: 7.4-12.2%) among 300 type 2 diabetic patients and 11.8% (8.8-14.8%) among 225 glucose tolerant control subjects (P=0.2). Among the 225 normal glucose tolerant subjects there was no difference in fasting values of glucose, insulin and C-peptide according to genotype. In contrast, after a 75 g oral glucose load carriers of the wildtype T/T -122 variant (N=175) had lower glucose levels (30 min: 7.7 mmol/l, 60 min: 7.1 mmol/l, 120 min: 5.4 mmol/l, $AUC_{0\text{-}120\ min}$: 177 minxmmol/l) compared to T/C -122 (N=48) and C/C -122 (N=2) carriers (30 min: 8.2 mmol/l, 60 min: 8.0 mmol/l, 120 min: 5.6 mmol/l, $AUC_{0\text{-}120\ min}$: 230 minxmmol/l) (P=0.037, P=0.011, P=0.27, P=0.010, respectively). No differences between carriers and non-carriers of the -122 variant in glucose stimulated insulin and C-peptide levels were found. In conclusion, we have identified a rare G/A -447 variant and a common T/C -122 variant in the promotor of the GLUT2 gene. The T/C -122 variant was not associated with type 2 diabetes but was significantly associated with increased plasma glucose levels up to 2 hours after an oral glucose tolerance test.

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INTERMEDIATE GAA REPEAT EXPANSIONS OF THE X25/FRATAXIN GENE IN TYPE 2 DIABETES.

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Type 2 (non-insulin dependent) diabetes mellitus is frequently associated with Friedreich's ataxia (FRDA). Homozygous expansion of > 66 repeat lengths of a GAA repeat in the 1st intron of the X25/frataxin gene is linked with the development of FRDA. Intermediate GAA expansions of 10-36 repeats do not lead to FRDA but were found to be increased in two white Caucasian Type 2 diabetic populations, although a subsequent comparable population association study failed to confirm this finding. **Aims:** given the potential problems of population association studies, we used a family based association method to investigate the importance of intermediate GAA expansions in Type 2 diabetes. **Materials and Methods:** the British Diabetic Association-Warren Trio collection comprises 170 family trios (Type 2 diabetic proband with both living parents) of North European extraction. Ascertainment was through the Type 2 diabetic probands, and MODY, Type 1 and 3243 mitochondrial diabetes were excluded by clinical, biochemical and genetic screening. The presence of the GAA repeats was determined using a PCR-based technique and separation of the products on a 1.5% agarose gel. **Results:** We have screened 104 families and detected 41 parents that are heterozygous carriers of intermediate expansion alleles. However, there is no evidence of preferential transmission of these alleles to diabetic offspring (22 vs 19 transmissions from heterozygous parents, NS). 22% of the diabetic probands are carriers of intermediate expansion alleles which is slightly lower than the rates of 24% and 27% detected in the population based studies. **Conclusions:** we have found no evidence to implicate intermediate GAA expansions of the X25/frataxin gene in the development of Type 2 diabetes in our population.

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***HFE* GENE HETEROGENETITY IN TYPE 2 DIABETIC PATIENTS WITH HAEMOCHROMATOSIS**

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Type 2 diabetes mellitus is reported in 50-72% of patients with haemochromatosis. This relatively high frequence has impelled several studies of prevalence of haemochromatosis in diabetic populations as well as mutational analysis of the candidate gene for hereditary haemochromatosis *(HFE)* among type 2 diabetic patients. These studies show that HFE mutations Cys282Tyr and His63Asp do occur in similar frequencies in patients with type 2 diabetes and in control subjects.

Aim: To study *HFE* gene in haemochromatosis patients detected by biochemical screening among type 2 diabetic patients and in hereditary haemochromatosis individuals.

Patients and methods: *Group A:* 24 patients with idiopathic haemochromatosis (17 men aged 37-65, mean 50; and 7 women, 29-70, mean 47). *Group B:* 5 patients from a group of 551 type 2 diabetic patients screened for haemochromatosis (275 men, mean 61.9; 276 women, mean 68). Screening criteria were transferrin saturation ≥ 50%, serum ferritin ≥ 1000 µg/l. In the absence of contraindications. Diagnosis was confirmed by liver biopsy in four of five and by total iron removed (in grams) >4g in the other.

Genomic DNA from all patients was extracted from peripheral blood. For detection of the Cys282Tyr and His63Asp mutations, genomic DNA was amplified by PCR and sequenced.

Results: The Cys282Tyr mutation was present on 87.5% of group A patients while no group B patient was homozygous for the mutation ($p<0.0005$). The frequency of the His63Asp mutation was the same in both groups.

There are no differences in serum ferritin or transferrin saturation values among both groups.

Conclusions: 1.) Haemochromatosis in type 2 diabetic patients is not clearly related to *HFE* gene. **2.)** Genetic heterogeneity has been found among idiopathic haemochromatosis patients. **3.)** There are no biochemical differences between haemochromatosis patients and type 2 diabetic individuals with haemocromatosis.

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ALDOSE REDUCTASE POLYMORPHISM IS NOT ASSOCIATED WITH RETINOPATHY IN TYPE II DIABETIC PATIENTS.

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Retinopathy is a common microvascular complication of diabetes. Prolonged hyperglycaemia and raised blood pressure are the main risk factors that contribute to the onset of retinopathy. Aldose Reductase (ALR2), is a key enzyme in the polyol pathway which converts D-glucose to sorbitol, the accumulation of which may contribute to retinopathy as well as cataract. A Short Tandem Repeat (STR) identified 2.1 kilobases upstream of the transcription initiation site of the ALR2 gene, has been associated with early onset retinopathy in a Chinese population (n=22 pairs) **Aims:** We examined the ALR2 STR and incidence of retinopathy in patients selected from the United Kingdom Prospective Diabetes Study (UKPDS). **Materials and Methods:** White Caucasian Type II Diabetics were matched pairwise (n=250 pairs), case (severe retinopathy requiring photocoagulation) to control (no retinopathy or just micro-aneurysms at same duration of known diabetes), for gender, age at diagnosis, fasting plasma glucose after 3 months diet, smoking, mean blood pressure 2 and 9 months after diagnosis. Fluorescent labelled primers were designed to amplify the STR by Polymerase Chain Reaction (PCR) and the products resolved by denaturing gel electrophoresis on an ABI 377 DNA sequencer. **Results:** No significant difference in allele or genotype frequency was detected between cases and controls ($p>0.1$). **Conclusion:** In this group of carefully matched patients selected from the UKPDS, there was no association of the ALR2 STR with retinopathy. This observation is in contrast to the Chinese study. Whether the discrepancy reflects ethnic differences remains to be clarified. Published data concerning the association of this polymorphism with nephropathy is also contradictory.

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CANDIDATE GENES FOR RISK AND/OR SEVERITY OF VASCULAR COMPLICATIONS OF DIABETES

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Aims: to investigate associations between common genetic polymorphisms and risk and/or severity of myocardial infarction (MI) or retinopathy (RP: by retinal photography, Wisconsin score) in non-insulin-dependent diabetes mellitus (NIDDM). **Materials & Methods:** approximately 350 cases with MI or RP and 350 matched controls were selected from the United Kingdom Prospective Diabetes Study. Controls were matched prospectively for gender, age, duration of disease, fasting plasma glucose, blood pressure, with the MI group also matched for smoking, and LDL and HDL cholesterol. The following common genetic polymorphisms were detected by PCR-based techniques: plasminogen activator inhibitor-1 (PAI-1) promoter 4G/5G, stromelysin (MMP3) promoter 5A/6A, tissue plasminogen activator (tPA) alu insertion/deletion and factor XIIIA P/L564. **Results:** Possession of the factor XIIIA L564 allele was significantly associated with RP (RR 1.9, 95% CI 1.1-2.4, n=542). Median Wisconsin retinopathy scores were 10 10 for the PP564 homozygotes, 20 10 for heterozygotes and 31 <31 for the LL564 homozygotes, with a significant test for trend. This polymorphism was not associated with MI. There was a borderline statistically significant association of homozygosity for the tPA insertion allele with risk of MI (RR 1.54, 95%CI 0.97-2.43, n=424) but not for angina. Cox proportional hazard analysis of determinants of risk of MI suggested that the tPA polymorphism may interact with the endothelial nitric oxide synthase IVS13 (CA)n and the paraoxonase Q/R192 polymorphisms ($p<0.02$). **Conclusions:** Common polymorphisms in tPA and factor XIIIA, both important factors in haemostasis and thrombosis, may predispose certain individuals with NIDDM to more severe RP or MI. This study also suggests that interactions between genetic polymorphisms may be important in determining risk and/or severity of vascular complications of NIDDM.

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CALCIUM/CALMODULIN DEPENDENT PROTEIN KINASE II β AND δ ARE HIGHLY EXPRESSED IN HUMAN β-CELLS

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The calcium/calmodulin dependent protein kinases II (CaM kinase II) are multifunctional enzymes which regulate gene expression, calcium homeostasis, and hormone and neurotransmitter release. In rats CaM kinase II isoforms α and β are exclusively expressed in neuronal tissues, while the isoforms γ and δ are expressed in neuronal and other tissues. Previous studies have shown, that CaM kinases II play an important role in glucose-stimulated insulin release. A dysfunction of this enzyme is thought to play a part in the multifunctional genesis of the type 2 diabetes mellitus. The **aim** of this study was the characterization of the CaM kinase II isoforms which are present in human β-cells, and which isoforms are predominantly expressed. **Methods:** A human insulinoma cDNA library was screened and several clones of the CaM kinase II β, γ and δ were detected. For RT-PCR primers of the isoforms β, γ and δ were designed according to the sequence of the cDNA clones, while a primer pair of the isoform α was created form a partial sequence published in GenBank. Total RNA from isolated human β-cells was reverse transcribed into cDNA and amplified by PCR. The expression levels of the CaM kinase II isoforms were determined by quantitative PCR using an internal DNA standard and by the comparison with the expression level of pyruvate dehydrogenase (PDH). **Results:** mRNA of the CaM kinase II isoforms β, γ and δ but not α were detected in human β-cells. The CaM kinase II β_3 subtype, previously described in human islets, was not found. The expression levels of the subtypes β and δ were equivalent, whereas the subtype γ was 10 fold lower expressed. The expression level of the housekeeping enzyme PDH was similar to the isoforms β and δ. **Conclusion:** The CaM kinase II isoforms β and δ are predominantly expressed in human β-cells at the same level of PDH. The high expression level of CaM kinases II β and δ indicate their important role in β-cell physiology.

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VITAMIN D RECEPTOR ALLELES AND VITAMIN D LEVELS IN GERMAN NIDDM PATIENTS.

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Alterations of vitamin D metabolism are well documented for experimental and human diabetes mellitus. Vitamin D deficiency impairs insulin release. Vitamin D receptors (VDR) are not only present in organs related to calcium metabolism but can be found in a variety of other tissues. VDR genotypes influence VDR gene transcription and/or mRNA stability, and common polymorphisms in the VDR gene are associated with IDDM and insulin secretion. Aims: To determine the impact of VDR polymorphisms in the pathogenesis of NIDDM. Materials and Methods: Vitamin D levels were studied together with VDR polymorphisms in 29 female and 43 male caucasian German patients with NIDDM. VDR genotypes were analysed from genomic DNA using PCR and restriction enzyme digestion with *Bsm*I, *Apa*I and *Taq*I. Vitamin D levels, HbA_{1c} as well as fasting and postprandial blood glucose, insulin and c-peptide were evaluated using commercially available kits. Results: The distribution of VDR genotypes among NIDDM patients (AA 40%, Aa 33%, aa 27%; BB 18%, Bb 52%, bb 30%; TT 35%, Tt 38%, tt 27%) did not differ from 200 matched controls and was also similar to controls reported by several American, Australian and French studies. However, the frequency of the b allele was significantly more frequent in patients when additional 192 patients with NIDDM were analysed for the *Bsm*I RFLP (58% vs. 51%, $p<0.03$%). There was no association of VDR polymorphisms with anti-diabetic medication, glycaemic control, insulin or c-peptide levels. Carriers of the bb genotype had significantly lower 25-hydroxyvitamin D levels than patients with the Bb genotype (bb 13.1±5.7ng/ml vs. Bb 19.0±7.8ng/ml, p=0.01, BB 16.7±6.3ng/ml). A similar, however, statistically not significant trend was found for calcitriol. Conclusions: The results do not rule out that VDR polymorphisms contribute to genetic susceptibility in NIDDM in the studied German population. Further studies on different ethnic populations and larger numbers are warranted to elucidate the influence of VDR polymorphisms on vitamin D levels and the pathogenesis of NIDDM.

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APO E POLYMORPHISM IS NOT ASSOCIATED WITH THE OCCURRENCE OF MACROVASCULAR COMPLICATIONS IN CZECH NIDDM PATIENTS

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Apolipoprotein E (apo E) plays a central role in the lipoprotein metabolism and polymorphism of the apo E gene is thought an important genetic factor for the development of macrovascular complications. Aims: We investigated the association of apo E genotypes (2/2, 2/3; 3/3; 3/4, 4/4 detected by Hha I digestion of PCR products) with lipid status and prevalence of macrovascular complications in patients with NIDDM (D, n=262) and the impact of polymorphism on lipid levels and glycemic control in juvenile hypertensives (HP, n=68) and controls (HC, n=66). Results: There were no signif. differences in the frequencies of apo E alleles and genotypes between the study groups which did not differ from Czech general population (GP,n=211), (χ^2 (0,05), n.s.). Serum total cholesterol (CH) and LDL-CH levels signif. differ according to apo E genotypes in D (Spearman corr. coef., CH: p=0039; LDL-CH: p=0,0116, ANOVA, CH: p=0,0008). The higher levels of CH, LDL-CH as well as HDL-CH and triacylglycerols were identified in persons with apo E 3/4 and 4/4, and were more pronounced in women (due to the positive average effects of ε4 allele on these variables in women). The higher occurrence of treated hypertension and ischemic heart disease in apo E 3/4 and 4/4 carriers was observed but it did not reach the stat. signif. There was also no correlation between apo E genotype and the occurrence of myocardial infarction, stroke, microalbuminuria, glucose control and any other screened metabolic parameter (χ^2 test, exact test of independence, Spearman corr. coef., ANOVA were used). Apo E was not signif. related to glucose control in juvenile hypertensives and controls, but those with apo E 2/3 tended to the lowest fasting glucose, fasting and stimulated C-peptide and insulin levels. Conclusions: In spite of the influence of apo E polymorphism on CH and LDL-CH levels our study showed no signif. differencies in the occurrence of macrovascular complications in patients with longterm NIDDM in relation to apo E genotype. *Supported by grants IGA MH 5395-5 and COST B5.*

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THE SST-I POLYMORPHISM AT THE APOC3 GENE LOCUS IN A POPULATION -BASED STUDY: ASSOCIATION WITH AN ATHEROGENIC LIPID PROFILE IN MEN.

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Aims: Apolipoprotein (apo)CIII is a major component of chylomicrons and very low density lipoproteins (VLDL), and is also present on high density lipoproteins (HDL). The APOC3 gene is clustered with the APOA1 and APOA4 genes on chromosome 11q23.
An Sst1 polymorphism on the 3' untranslated region of the APOC3 gene has been described and the rare allele (S2) has been associated with higher triglycerides (TG), total cholesterol (TC), and apo CIII plasma levels on several, but not all, studies. The aim of this study was to assess whether the S2 allele has a significant effect on plasma lipids and coronary heart disease (CHD) risk in a large population-based study: The Framingham Offspring Study.
Materials and Methods: The SstI polymorphism was examined in 2485 study participants (1219 males, 1266 females) using PCR and restriction endonuclease analysis with SstI.
Results: The frequency of the S2 allele was 0.09, consistent with previous reports in Caucasian populations. We found a gender specific association between the presence of the S2 allele and plasma lipid concentrations. In men, the S2 allele was associated with significantly lower HDL cholesterol ($p<0.04$) and HDL2 cholesterol ($p<0.02$) levels; whereas in women the association was observed with increased total-($p<0.03$), LDL-cholesterol ($p<0.03$) and apo B levels ($p<0.04$). The previously reported association of the S2 allele with higher TG and apo CIII levels was noted only in men, but it did not reach statistical significance. However, S2 male carriers had significantly smaller particle size ($p<0.04$), as assessed by nuclear magnetic resonance. The presence of the S2 allele did not affect CHD risk or the age of onset in this normal population.
Conclusions: The associations of the S2 allele at the APOC3 locus with lower HDL-C, increased TG levels and smaller LDL size observed in males, suggest that this genetic variant could exacerbate the atherogenic lipoprotein profile characteristic of diabetic subjects, thus increasing their atherogenic risk.

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ANALYSIS OF THE NKX 2.2 GENE FOR MUTATIONS IN PATIENTS WITH LATE ONSET TYPE 2 DIABETES

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Aim. Since disruption of the NKX 2.2 gene in mice results in congenital diabetes we tested the hypothesis that variability of the human NKX 2.2 gene is associated with subsets of type 2 diabetes
Methods and Materials. The coding region of the NKX 2.2 gene was examined by combined single strand conformational polymorphism - heteroduplex analysis of genomic DNA from 244 Danish Caucasian patients with late onset type 2 diabetes. Two of the three identified variants were subjected to association studies comprising the 244 diabetic patients and 236 matched glucose tolerant control subjects.
Results.

	Allelic frequency	
Variant	Diabetic patients	- Glucose tolerant controls
P19Q (ccg/cag)	0,002	Not analysed
A42T (gcc/acc)	0,08	0,07
G152A (ggg/gcg)	0,03	0,02

The A42T and G152A polymorphisms were not associated with diabetes. The P19Q mutation was detected in only one family comprising 3 generations. The mutation however, did not segregate with diabetes nor did it affect the age of diabetes onset.
In conclusion. We have identified amino acid substitutions in the coding region of the NKX 2.2 gene. None of them are associated with late onset type 2 diabetes among Danish Caucasians.

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CLINICAL CHARACTERISTICS OF TYPE 2 DIABETES MELLITUS THAT IS LINKED WITH CHROMOSOME 20q

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Aims: The report of a genome scan for linkage with obesity, an intervening phenotype for type 2 diabetes, described evidence for an obesity gene in the region of chromosome 20q where we previously found evidence for linkage with type 2 diabetes mellitus in Caucasians. Our finding has been confirmed in several other family collections. This report describes the phenotype of the diabetes linked with this region and examines the region for evidence in our families for linkage with obesity. **Materials and Methods**: Our family collection has grown from 29 to 43 families and includes 526 genotyped individuals, 263 with diabetes. Fifteen microsatellite markers spanning the entire length of chromosome 20 were genotyped. Nonparametric linkage analyses for type 2 diabetes and obesity (defined as BMI>30.0) were done by GENEHUNTER. **Results:** We found linkage with type 2 diabetes at a single region on chromosome 20q. The maximum NPL score (4.1, $p=5.0x10^{-4}$), corresponding to a lod score 3.51 ($Z^2/2\ln(10)$), for all families occurred at marker D20S196. There was evidence of heterogeneity as 7 families had a maximum NPL scores over 2.1 (lod >1.0). The 43 diabetic patients in these 7 families were characterized by a middle to late age at onset of diabetes (mean=46 years) and obesity (mean BMI= 30.1). In these regards, they were indistinguishable from the 223 affected individuals in the remaining 36 families. Both groups were characterized by fasting and post-challenge hyperinsulinemia. We found no evidence for linkage of markers on chromosome 20q with obesity. **Conclusions:** We provide further evidence for strong linkage between type 2 diabetes and a specific region on chromosome 20q. Diabetes in the linked and unlinked families was similar with regard to age at onset, obesity, fasting and post-challenge insulin levels. The recently described obesity locus on chromosome 20q does not contribute to the development of type 2 diabetes in our family collection.

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DETECTION OF A NEW VARIANT IN THE MITOCHONDRIAL GLYCEROL PHOSPHATE DEHYDROGENASE GENE IN SPANISH TYPE 2 DM PATIENTS.

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Screening for mutations in the FAD-linked glycerolphosphate dehydrogenase gene (mGPDH) has yielded positive results in some individuals with type 2 DM. Our recently findings. have shown the existence of mutations affecting the amino-acid sequence of the FAD binding-domain in one type 2 DM patient. The **aim** of the present study was to evaluate if defects in the FAD binding-domain of the mGPDH gene could contribute to the susceptibility to type 2 DM. **Methods:** Human genomic DNA was extracted from peripheral blood leukocytes. Screening by single strand conformational polymorphism (SSCP) was carried out on 151 type 2 DM patients and 139 non diabetic control Caucasian subjects. The nucleotide changes corresponding to each abnormal SSCP were determined by direct sequencing. **Results:** An electrophoretic variant pattern was detected by SSCP screening of the mGPDH gene in six subjects (5 type 2 DM subjects / 1 control subject). Direct sequencing of the DNA fragments with the variable pattern revealed the same mutations in the intron-3: A single substitution (T-A) at position 18 and 6 base pair deletion situated at position 26. These nucleotides changes were found in heterozygous form. Subjects with the intron mutation compared to those type 2 DM subjects without the mutation, were older (69.8 ± 1.5 vs. 62.7 ± 13.2, $p< 0.001$) and the evolution time of their diabetes was longer (24.2 ± 11.1 vs. 12.6 ± 8.7, $p< 0.005$). Members of the proband's families were also studied. Only one of these studied families showed cosegregation. A part from the proband, two women (his sister an his niece) who have had a gestational diabetes shared the same pattern. We have not further observed those mutations previously found affecting the amino-acid sequence of the FAD-binding-domain. **Conclusions**: New mutations in the mGPDH gene are described in type 2 diabetic population. This intron mutation are not in the known consensus sequence for splicing, however we can not role out its functional significance in the correct mechanism of the FAD-binding-domain mRNA transcription.

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HETEROPLASMY LEVELS OF THE A3243G MUTATION, HLA DQ POLYMORPHISM AND PHENOTYPE OF MATERNALLY INHERITED DIABETES AND DEAFNESS.

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Aims: Maternally Inherited Diabetes and Deafness (MIDD) associates with a mutation at position 3243 in mitochondrial DNA. Phenotypic expression of MIDD includes type 1 and type 2-like diabetes. We examined whether the degree of heteroplasmy in leukocyte and oral mucosa DNA and HLA haplotypes influence clinical expression of the 3243 mutation.
Materials and Methods: In a group of 20 unrelated probands with MIDD, eight having type 1-like diabetes, twelve with type 2-like diabetes, HLA DQ and heteroplasmy levels for the 3243 mutation were determined. HLA DQA1/DQB1 phenotypes were categorised as predisposing, neutral or protective for autoimmune-mediated Type 1 diabetes.
Results: No differences were observed between type 1 and type 2-like MIDD groups with respect to the cumulative frequency of protective and predisposing haplotypes. Predisposing HLA-DQ types are more prevalent in MIDD patients than in autoimmune type 1 diabetic patients ($p<0.05$). Heteroplasmy levels for the 3243 mutation showed large variations in patients, ranging from 1% to 52% in leukocyte DNA. A strong relationship was seen between heteroplasmy values in leukocyte DNA and DNA from oral mucosa cells ($r=0.89$, $p<0.001$). No correlation was observed between the degree of heteroplasmy and diabetic phenotype, even when group size was extended with diabetic relatives of patients with MIDD. The onset age of diabetes was not correlated with heteroplasmy levels, but heteroplasmy levels decreased with age.
Conclusions: We conclude that the phenotype of diabetes in MIDD is independent of HLA haplotype and independent of the degree of heteroplasmy indicating that other, as yet unknown, factors modulate clinical expression of the A3243G mitochondrial mutation.

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MITOCHONDRIAL DNA MUTATIONS ARE ASSOCIATED WITH BOTH DECREASED INSULIN SECRETION AND PROGRESSION OF MICROVASCULAR COMPLICATIONS IN JAPANESE DIABETIC SUBJECTS

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Aims: To assess the roles of mitochondrial (Mt) DNA mutations in diabetic and nondiabetic subjects, we screened Mt DNAs at the 3243 base pair (bp) and its adjacent portion in diabetic and nondiabetic subjects. Furthermore, to clarify the clinical features of diabetic subjects harboring an Mt DNA mutation, we evaluated the ability of insulin secretion and microvascular complications in diabetic subjects.

Materials and Methods: Baseline characteristics in unrelated 537 diabetic subjects and 612 nondiabetic subjects were exanined, and microvascular complications and insulin secretory capacity in diabetic subjects were newly evaluated. Mt DNAs isolated from peripheral leukocytes were analyzed by PCR.

Results: Eight kinds (np 3243, 3316, 3394, 3714, 3593, 3639, 3391, and 3537) of the Mt DNA mutations found in 74 subjects were point mutations. The numbers of affected diabetic and nondiabetic subjects were 45 (8.4%) and 29 (4.7%)($p=0.012$), respectively. There was no difference in the prevalence of maternally inherited diabetes between these two groups. The mean level of urinary C-peptide excretion was lower in diabetic subjects with an Mt DNA mutation (DM+) than in those without it (DM-). Diabetic retinopathy and nephropathy in DM+ were serious, in comparison with those in DM-. Furthermore, a logistic regression analysis revealed that advanced retinopathy and decreased urinary C-peptide excretion in all diabetic subjects studied were strongly related to the presence of Mt DNA mutation.

Conclusions: Our results suggest that Mt DNA mutations are related to the development of diabetes, and also that these mutations are associated with not only a decrease in insulin secretion but also progression of diabetic microvascular complications.

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ACCURATE DETECTION OF LOW PERCENTAGES OF MUTATION A3243G IN BLOOD USING A NEW NON-RADIOACTIVE METHOD.

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Aims: The A3243G mitochondrial DNA mutation, the most common cause for mitochondrial diabetes mellitus, is usually detected in blood using PCR, *Apa I* restriction enzyme analysis and agarose ethidium bromide staining. This is a simple but insensitive method. However, sensitive screening procedures for mtDNA mutations must be used for testing patients diagnosed with only diabetes mellitus, who usually carry low levels of heteroplasmic DNA mutations. The most sensitive method to date is based on autoradiography, but radioactive methods are expensive, lengthy and prohibited in some laboratories. Consequently the aim of the study was to test a new non-radioactive method of detection for the mitochondrial A3243G mutation. **Materials and Methods:** DNA samples were collected from 50 diabetic patients with deafness and/or maternally inherited diabetes mellitus. The same *Apa I* digests were firstly revealed by standard ethidium bromide staining and secondly by 6% polyacrylamide gel electrophoresis stained with silver. **Results:** Using standard method, A3243G mutation was detected in only 4 patients. Using polyacrylamide silver staining, A3243G mutation was clearly identifiable in 6 patients. Furthermore, when only a faint band was visible for one patient's undiluted PCR-product after standard method, the same sample diluted with 1/8 was clearly identifiable using polyacrylamide silver staining. **Conclusions:** We suggest that the non-radioactive method based on 6% polyacrylamide gel electrophoresis stained with silver could be more sensitive than the standard method in identifying the A3243G mutation and the other known mitochondrial DNA mutations.

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Signal Transduction in β-Cells

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CHARACTERISATION OF GLUCOKINASE INTERACTING PROTEINS TROUGH A PHAGE DISPLAY PEPTIDE LIBRARY

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Aims: The glucose recognition enzyme glucokinase (GK) is regulated in liver through an interaction with a regulatory protein (GRP). It was the aim of this study to identify potential interacting proteins of glucokinase by a systematic approach which may have a glucokinase regulatory function in pancreatic beta cells. **Methods**: Recombinant human beta cell glucokinase was fixed to a solid support by a His-Tag and screened for interaction with a phage display peptide library of 12-mer random peptides fused to a minor coat protein pIII of the M13 phage. Through biopanning steps of high stringency specific GK-interacting peptide sequences were enriched. GK interaction of the consensus peptides and complete proteins was verified by ELISA sandwich assays, protein binding assays and the yeast GAL4 two-hybrid system. **Results**: Two proteins showed a strong interaction with human beta cell GK: 1. The GRP of the liver and 2. the bifunctional glycolytic enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK II) with a consensus motif of six amino acids corresponding to a ß-sheet of the fructose-2,6-bisphosphatase domain. Binding studies and two-hybrid assays revealed that the binding is specific for GK and that the interaction of GRP with GK is stronger than that of PFK II. **Conclusion**: The interaction of GK with the glycolytic enzyme PFK II may provide a possible mechanism of posttranslational regulation of GK in pancreatic beta cells through formation of a multienzyme complex which favours metabolic channeling as well as modulation of GK enzyme activity. The GK/PFK II complex may trigger oscillatory metabolic phenomena in pancreatic beta cells which have a significant impact on stimulus-secretion coupling.

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IMPORTANCE OF CYSTEINE RESIDUES FOR THE STABILITY AND CATALYTIC ACTIVITY OF HUMAN GLUCOKINASE

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Aims: The glucose phosphorylating enzyme glucokinase is the glucose sensor in pancreatic beta cells. It was the aim of the study to characterise the importance of cysteine residues for the catalytic function using the SH group reagent alloxan as a tool. **Methods:** Wild-type and mutant human beta cell glucokinase protein was expressed in E. coli bacteria and purified by metal chelate chromatography. Formation of intramolecular disulphide bridges was shown through the electrophoretic band pattern after controlled proteolysis by proteinase K. Cysteine residues (C) were exchanged by serine through site-directed mutagenesis. **Results:** Incubation of glucokinase with alloxan induced the formation of multiple intramolecular disulphide bridges corresponding to a double band pattern (58 and 49 kDa) in non-reducing SDS PAGE due to different conformations of the protein. Mutation of cysteine residues could not prevent the formation of the 49 kDa glucokinase species after alloxan treatment. The cysteine mutants C233, C252 and C382 showed a complete loss of catalytic activity. The V_{max} values of the C213, C220, C364 and C371 mutants for glucose and mannose were significantly ($p < 0.01$) decreased by 30–60 % whereas the C230 mutant showed kinetic characteristics comparable to that of wild-type glucokinase. The sensitivity of the C213, C230, C364 and C371 mutants towards an alloxan-induced inhibition of enzyme activity was up to tenfold lower ($p < 0.01$) compared to wild-type glucokinase. **Conclusion:** The data provide evidence that multiple intramolecular disulphide bridges contribute to the well-known instability of the glucokinase enzyme. The identification of sensitive cysteine targets may help to develop strategies which protect glucokinase against sulfhydryl attack and oxidative stress. In particular the C230 mutant may serve as a glucose sensor with an improved stability in insulin-secreting surrogate cells for diabetes gene therapy.

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IDENTIFICATION OF A NOVEL GLUCOSE-STIMULATED, STRESS-ACTIVATED KINASE IN PANCREATIC ISLETS.

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AIMS: Elevated extracellular glucose concentrations may stimulate the transcription of preproinsulin and other genes through the activation of stress-activated protein kinases (SAPKs) including SAPK2 (also called p38 or reactivating kinase, RK). In-gel kinase assay was used to measure the activation of SAPK2, and identify novel glucose regulated members of this family. **Materials and methods:** The transcription factor and SAPK target Activating Transcription Factor 2 (ATF2) was immobilised on 10% SDS-PAGE gels as a fusion protein with Glutathione-S-Transferase (GST-ATF2). Total cell extracts from glucose-, UV- or TPA-stimulated rat islets of Langerhans were subjected to separation on the substrate-containing gel and subsequently exposed to γ-^{32}P-ATP. Activation of the kinases was quantitated via measurements of ^{32}P incorporated into individual bands. **Results:** Whilst SAPK2 was poorly (~1.5 fold) stimulated by incubation of islets at elevated glucose concentrations, the activity of an ATF2 kinase of relative molecular mass close to 63 kDa (p63) was stimulated ~7 fold by 16.6 versus 3mM glucose. This activation, which was maximal after 15 min., could be mimicked by cellular stresses including UV irradiation, but was markedly smaller at high glucose concentrations (> 30mM). **Conclusions:** p63 may have an important role in signal transduction between glucose metabolism and gene expression, some of which have previously been attributed to SAPK2.

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INVOLVEMENT OF MAPKAP KINASE-2 IN IL-1ß INDUCTION OF NITRIC OXIDE SYNTHASE IN HIT-T15 CELLS

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Preliminary studies using an inhibitor of p38 MAPK, suggest a role for this kinase in IL-1ß signalling. A potential candidate for downstream signalling events is MAPKAP kinase-2 which is known to be phosphorylated by p38. **Aims:** To use transfection, in addition to inhibitor studies, to determine the potential role of MAPKAP kinase-2 as an effector for p38 during IL-1ß induction of nitric oxide synthase (iNOS) in HIT-T15 cells. **Materials and Methods:** HIT cells were transiently transfected with a catalytically inactive (dominant negative, DN) mutant of murine MAPKAP kinase-2 or control vector (pCR™3) alone. After 48h incubation with IL-1ß (100pmol/l), insulin secretion, nitrite formation and iNOS protein expression were determined. **Results:** Transfected cells express the DN MAPKAP kinase-2 as determined by western blotting for c-myc which is tagged to the gene. IL-1ß treatment decreased glucose-induced insulin secretion in untransfected and control-transfected cells. IL-1ß inhibition of insulin secretion was not reversed by transfection with the DN MAPKAP kinase-2. However, IL-1ß induction of nitrite formation was partially reversed in cells expressing the DN MAPKAP kinase-2, compared to cells transfected with the vector alone (from 17.9±0.9 to 14.3±0.2µmol/l; $p<0.01$). These results are in agreement with those obtained using the p38MAPK inhibitor, SB203580 (50µmol/l). Western blotting for iNOS similarly showed reduced expression of the enzyme (by 50%) in cells transfected with the DN construct. **Conclusions:** Transient transfection experiments utilising a dominant negative kinase construct suggest that IL-1ß induction of nitric oxide synthase may be mediated partly by MAPKAP kinase-2.

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PANCREATIC β-CELL PROLIFERATION INVOLVES P42/44 MITOGEN-ACTIVATED PROTEIN KINASES

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AIMS: We have previously identified the p42/44 mitogen-activated protein kinases (MAPKs) in adult rat islets and the MIN6 β-cell line, and have demonstrated that signalling through these enzymes is not required for regulated insulin release. Since MAPKs have been shown to be involved in the transduction of mitogenic signals in other cell types, the aim of this study was to investigate the involvement of p42/44 MAPKs in the proliferation of the MIN6 β-cell line. **METHODS AND RESULTS:** Exposure of 24h serum-starved MIN6 β-cells to 1-15% (v/v) foetal calf serum (FCS) for 24h induced significant increases in β-cell proliferation, as assessed by colorimetric detection of bromodeoxyuridine (BrdU) incorporation into newly synthesised DNA using an HRP-coupled anti-BrdU antibody (0% FCS, 100±4.9%; 1% FCS, 173±14.3; 2% FCS, 245±13.1%; 15% FCS, 228±18.8, $P<0.001$ vs 0% FCS, n=6). Inhibition of p42/44 MAPK activities by PD098059 (PD; 20µM, 4h) significantly inhibited FCS-stimulated MIN6 β-cell proliferation (53±7% FCS-stimulated, $P<0.001$, n=6). BrdU incorporation was also assessed by fluorescence immunocytochemistry, using a FITC-conjugated anti-BrdU antibody to detect BrdU incorporation. In these experiments PD (50 or 100µM) also significantly reduced the levels of FCS-stimulated BrdU incorporation into MIN6 β-cell DNA, with a more marked inhibitory effect at 100µM (50µM PD, $P<0.05$; 100µM, $P<0.01$). PD, at concentrations up to 100µM, had little effect on cell appearance or cell viability as assessed by measuring Trypan blue uptake. **CONCLUSION:** These data implicate signalling through the p42/44 MAPK pathways in the regulation of MIN6 β-cell proliferation.

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ATF-2 MEDIATES SIGNAL TRANSDUCTION FROM Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE IV IN INSULIN GENE EXPRESSION

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We previously reported that the transcriptional factor ATF-2 binds to the cyclic AMP-responsive elements (CREs) of the human insulin gene. Here, we report that ATF-2 enhances glucose-induced insulin promoter activity when transfected into rat primary cultured islets, while CREB represses it. The transcriptional activity of ATF-2 was enhanced by Ca^{2+}/calmodulin-dependent protein kinase IV (CaMKIV), but not by cyclic AMP-dependent protein kinase (PKA) or Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). Mutagenesis study showed that the Thr^{69}, Thr^{71}, and Thr^{73} of ATF-2 are necessary for the activation by CaMKIV. The CaMKIV-induced ATF-2 transactivity was not mediated by the activation of c-Jun NH_2-terminal protein kinase (JNK) or p38 MAP kinase. Co-transfection with CBP or p300 expression plasmids further increased the transcriptional activity of ATF-2 induced by CaMKIV. Transcription of the human insulin gene, enhanced by the elevated intracellular concentration of calcium ions, was completely blocked by Ca^{2+}/calmodulin-dependent protein kinase inhibitor. These results suggest that ATF-2 increases insulin gene expression in pancreatic β-cells through activation by CaMKIV.

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GLUCOKINASE-SENSITIVE GLUCOSE METABOLISM IN INS-1 CELLS OVEREXPRESSING LACTATE DEHYDROGENASE

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Aims: The channelling of glucose metabolic flux towards mitochondrial oxidation is critical for initiation of insulin secretion. However, the precise regulatory principles in the control of oxidative versus anaerobic glycolysis are still unresolved. It was the aim of this study to characterise the role of lactate dehydrogenase (LDH) as a metabolic modulator in insulin-secreting INS-1 cells. **Methods:** The M-isoform of LDH was stably transfected in INS-1 cells. Cell clones with a 20-fold and 5-fold overexpression of LDH were selected through their mRNA, protein and enzyme levels. The dichotomy of anaerobic and aerobic glucose metabolism was measured by the production of $^{14}CO_2$ from D-[U-^{14}C]glucose and lactate release into the medium. **Results:** INS-1 cells with a 20-fold overexpression of LDH showed a 40 % decrease of the glucose oxidation rate at high glucose concentration (16.7 mM) compared with control cells ($p < 0.05$) whereas glucose oxidation at 1 mM glucose was only slightly reduced by 15 %. Lactate release was increased by 200 % at 16.7 mM glucose and 80 % at 1 mM glucose (20-fold LDH overexpressing INS-1 cells vs. control cells). 5-fold overexpression of LDH resulted in a 30 % decrease of glucose oxidation at 16.7 mM glucose compared with controls ($p < 0.05$) whereas no difference was observed at 1 mM glucose. Lactate release was significantly increased (70 %) only at 16.7 mM. Nevertheless, the insulin secretory response was not significantly modified by the overexpression of LDH. **Conclusion:** The data provide evidence that glucokinase-mediated but not hexokinase-mediated glycolytic flux is highly sensitive towards anaerobic lactate production by LDH. The preferential channelling of aerobic glucose metabolism by glucokinase in pancreatic beta cells may explain the low constitutive expression levels of LDH.

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POTENTIAL GENES INVOLVED IN HUMAN β-CELL GROWTH STIMULATION AFTER UNI-LATERAL NEPHRECTOMY

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We have previously shown that the proliferative activity of adult human β-cells is quite limited, but, nevertheless, glucose stimulates human β-cell proliferation *in vitro*. We have also shown that mouse islet cell proliferation in a subcapsular renal graft is considerably stimulated, when the recipient is nephrectomized. **Aims:** To investigate whether transplanted human β-cells increase their proliferative activity when stimulated by growth factors released after uni-lateral nephrectomy, and to explore which islet cell genes are differentially expressed after such a stimulation. **Methods:** Human islets, prepared from a total of 8 heart-beating organ donors (β-cell Transplant, Brussels), were transplanted (day 0) under the kidney capsule of nude C57bl/6J mice. The recipients were either sham-operated or uni-laterally nephrectomized on day 14. For cell proliferation analysis, a BrdU tablet was implanted s.c. in the neck region at the same time as the nephrectomy, or BrdU was injected 2h prior to killing of the mice on day 15, 17 or 19. The labelling index (LI; i.e. % of labelled cells over total number of cells) for β-cells was estimated by light microscopy after double immunohistochemistry for insulin and BrdU. For differential display, grafts were harvested on day 15 and 17 and total RNA was extracted. RT-PCR was performed with random primer pairs and the results displayed on large polyacrylamide gels, where differentially displayed bands could be examined. **Results:** In uni-laterally nephrectomized mice, human β-cells transplanted under the kidney capsule were affected by the growth stimulation and increased their proliferative rate with 190% compared to sham-operated controls, during a 5 day period (0.30±0.06 vs. 0.12±0.04; $p<0.01$ paired t-test). After pulse-labelling with BrdU, β-cell LI was increased with 50% compared to controls 3 days after nephrectomy, whereas LI had not yet begun to increase 1 day after nephrectomy. Preliminary results show that 1 and 3 days after nephrectomy 2 genes were differentially displayed, whereas 2 other genes were no longer expressed compared to sham-operated controls. **Conclusions:** The present experimental model is probably the first in which human β-cell growth stimulation is both induced and experimentally monitored. Four genes potentially involved in human islet-cell proliferation have been identified and are currently being sequenced and further investigated.

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THE NOVEL HYPOGLYCEMIC AGENT JTT-608 IS A PHOSPHODIESTERASE INHIBITOR

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Aims: We studied the effects of the novel hypoglycemic agent JTT-608 [trans-4-(4-methylcyclohexyl)-4-oxobutyric acid] on insulin secretion from pancreatic islets, and analyzed the mechanism of its effect. **Methods:** Insulin secretory capacity by static incubation and in perifusion conditions from isolated intact islets, Ca^{2+}-induced insulin secretion from electrically permeabilized islets, and the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) by fluorescence measurement were examined. **Results:** JTT-608 augmented 8.3 mM glucose-induced insulin secretion dose-dependently, and there was a stimulatory effect of 100 μM JTT-608 at both moderate and high concentrations (8.3, 11.1 and 16.7 mM) of glucose but not at low concentrations (3.3 and 5.5 mM). In perifusion experiments, both phases of insulin release were enhanced, and the effect was eliminated 10 min after withdrawal of the agent. In the presence of 200 μM diazoxide and a depolarizing concentration (30 mM) of K^+, there was an augmentation of insulin secretion by 100 μM JTT-608, not only under high levels of glucose but also under low levels, and the effects were abolished by 10 μM nitrendipine. JTT-608 augmented insulin secretion from electrically permeabilized islets in the presence of stimulatory concentrations (0.3 and 1.0 μM) of Ca^{2+}, and $[Ca^{2+}]_i$ response under 16.7 mM glucose, 200 μM diazoxide, and 30 mM K^+ also was augmented. In addition, the cAMP content in the islets was increased by 100 μM JTT-608, and a synergic effect with 1 μM forskolin was observed, but not in the presence of 50 μM 3-isobuthyl-1-methylxanthine. JTT-608 was found to inhibit phosphodiesterase (PDE) activity dose-dependently. **Conclusions:** We conclude that JTT-608 augments insulin secretion by enhancing Ca^{2+} efficacy and by increasing Ca^{2+} influx which is a result of the increased intracellular cAMP concentration due to PDE inhibition.

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Metabolic Signalling

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D-GLUCOSE METABOLISM IN RAT ISLETS EXPOSED TO DIAZOXIDE AND HIGH K^+ CONCENTRATIONS

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Aims: Even in the presence of diazoxide, D-glucose stimulates insulin release provided that the B-cell is exposed to a high concentration of K^+. The metabolism of the hexose was now investigated in isolated islets incubated under such experimental conditions. **Methods:** The metabolism of D-[5-^{3}H]glucose and D-[U-^{14}C]glucose was measured in islets from fed rats over 120 min incubation. **Results:** At high (16.7 mmol/l) but not low (5.6 mmol/l) D-glucose concentration, diazoxide (0.25 mmol/l) decreased the paired ratio between $^{14}CO_2$ and ^{3}HOH generation, this coinciding with a higher net production of ^{14}C-labelled amino acids and acidic metabolites. Whether in the absence or presence of diazoxide, equimolar substitution of NaCl by KCl, to raise the K^+ concentration from 5 to 90 mmol/l, decreased modestly, but significantly, both the oxidation of D-[U-^{14}C]glucose and utilization of D-[5-^{3}H]glucose, at least at high concentrations of the hexose (8.3 and 16.7 mmol/l). At a normal K^+ concentration, the absence of Ca^{2+} (no $CaCl_2$, EGTA 1 mmol/l) decreased the production of ^{3}HOH and, even more so, that of $^{14}CO_2$, these changes being again most marked at a high concentration of D-glucose. An inhibition of the catabolism of D-glucose (16.7 mmol/l) attributable to Ca^{2+} deprivation was also documented in islets exposed to a high K^+ concentration (90 mmol/l) and diazoxide. **Conclusions:** Whilst confirming the key role of cellular Ca^{2+} accumulation in the preferential stimulation of oxidative events in glucose-stimulated islets, these findings indicate that the experimental conditions currently used to investigate the component of D-glucose insulinotropic action not attributable to the closing of ATP-sensitive K^+ channels only cause a modest impairment of its catabolism in islet cells.

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Does biphasic insulin secretion require Krebs-cycle anaplerosis ?.

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Aims: We have tested the hypothesis that a sustained stimulation of insulin secretion requires not only feeding the Krebs cycle with acetyl-CoA but also the anaplerotic replenishment of cycle intermediates.**Materials and methods:** For that purpose, isolated rat islets were perifused with substrates that are metabolized exclusively to acetyl-CoA (10 mmol/l L-leucine) and to a cycle intermediate (10 mmol/l L-glutamine, succinic acid dimethyl ester or SAD, and D-glucose), and the secretion of insulin was radioimmunologically measured (ng/min x 40 islets) in the effluent every minute. **Results:** L-leucine induced a transient, monophasic stimulation of insulin secretion that lasted for 10-15 minutes. Depolarization with 30 mmol/l K^+ mimicked L-leucine stimulation. L-glutamine, which is not a secretagogue by itself, did not significantly modify depolarization-induced secretion, even in the presence of 3 mmol/l glucose (0.40 ±0.06,n=13 vs. 0.33 ±0.06,n=11;N.S.). However, it induced a second phase of release, after the first phase initiated by L-leucine, that doubled the the rate of secretion (0.72 ±0.09,n=12 vs. 0.32 ±0.04,n=11;p<0.01). A subthreshold glucose concentration (3 mmol/l) could partially substitute for L-glutamine and sustain in time the transient secretory effect of L-leucine (0.56 ±0.1, n=7 vs. 0.32 ±0.04,n=11;p<0.05).This effect was even more pronunced at 6 mmol/l glucose (0.92 ±0.1,n=13 vs. 0.32 ±0.04,n=11;p<0.001). SAD alone triggered a biphasic insulin secretion at 10 mmol/l that was not significantly modified by L-leucine (0.71 ±0.15,n=9 vs. 0.83 ± 0.1,n=10;N.S.) and was lower than that induced by a maximum glucose stimulus. At 3 mmol/l, SAD stimulated a low, but sustained, rate of insulin secretion that was significantly increased in the presence of L-leucine (0.18 ±0.02,n=9 vs. 0.44 ±0.04,n=8;p<0.001).**Conclusions:** The fact that the monophasic stimulation of insulin secretion by L-leucine is made biphasic by L-glutamine, and low concentrations of glucose or SAD, supports the need of the anaplerotic replenishment of Krebs cycle intermediates for a sustained secretion.

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POTENTIATION OF GLUCOSE-INDUCED INSULIN RELEASE WITHOUT CORRESPONDING CHANGES IN OXYGEN CONSUMPTION

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The islet respiratory response to a rise in glucose is one of the primary triggering factors leading to insulin release. This response varies between islets under physiological conditions. The corresponding insulin secretion of the islets via this Ca^{2+}-dependent pathway is, however, tightly coupled to the inter-islet variation in the respiratory response. It is not know if stimulation of insulin release via the Ca^{2+}-independent pathway is correlated to changes in oxygen consumption. **Aim**: We have examined the respiratory and secretory responses of individual islets stimulated by activators of proteinkinase A and C. **Materials and methods**: Oxygen tension (pO_2) and insulin release were measured simultaneously from individual islets isolated from the ob/ob mouse. The islets were perifused at 3 or 11 mM glucose either at 1.28 mM Ca^{2+} or in a Ca^{2+} -deficient medium (0.5 mM EGTA) in the presence or absence of 100 nM phorbol myristate acetate (PMA) and 10 µM forskolin. **Results**: Similar glucose-induced decreases in pO_2 were observed both with Ca^{2+} -containing and –deficient media in the absence or presence of PMA and forskolin. When decrease in pO_2 was plotted against insulin release at 11 mM glucose for individual islets, the correlation obtained under physiological conditions (r^2=0.95 p<0.05) was no longer present when islets were perifused with Ca^{2+}-deficient medium, which inhibited insulin release. Although insulin release was restored to levels obtained under physiological conditions when PMA and forskolin were present in Ca^{2+}-deficient medium, no correlation was found between respiratory and secretory changes in the individual islets. Also, when PMA and forskolin were present in Ca^{2+}-containing medium there was no correlation between respiratory changes and the 3-fold increase in insulin release induced by the activators. **Conclusion**: Activation of PKA and PKC potentiates glucose-induced insulin release from individual islets without corresponding changes in respiration.

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2-DEOXYGLUCOSE INHIBITS GLUCOSE METABOLISM BUT NOT INSULIN SECRETION IN HIT-T15 CELLS IN NUTRIENT-RICH DMEM MEDIUM.

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Aims: To investigate the effects of inhibitors of glycolysis on glucose-stimulated insulin secretion (GSIS) in phosphate buffered saline (PBS), and a medium (DMEM) containing exogenous nutrients other than glucose (such as amino acids).

Materials and Methods: We employed multi-nuclear NMR spectroscopy, the perforated patch-clamp method, and conventional enzymatic and insulin assays to study the effects of 2-deoxyglucose (2-DG) on glucose metabolism, cellular bioenergetics, K_{ATP} current, and GSIS in the glucose responsive HIT-T15 cell line.

Results: In DMEM and in the presence of 20mM glucose (G20), 2-DG treatment (20mM) reduced the glucose utilization rate by 72±5%, completely blocked the incorporation of [1-^{13}C] glucose into [3-^{13}C] lactate, reduced its incorporation into [4-^{13}C] glutamate by 56 ± 3 %, decreased the cellular ATP and the ATP/ADP ratio by 30±3 and 70±3 respectively, activated K_{ATP} channels by 82±18% but failed to inhibit GSIS. Similar behavior was observed in PBS with the notable exception of GSIS, which was inhibited. Furthermore, 2-DG markedly opened K_{ATP} channels in G0-DMEM (by 195±6%), suggesting that it may act on the channel independently of its inhibition of glucose metabolism. The effect of 2-DG was not observed in G0-PBS, exemplifying the importance of incubation media on experimental outcome and conclusions. All reported effects were statistically significant (p<0.01).

Conclusions: The dissociation of GSIS from glucose metabolism, ATP, the ATP/ADP ratio, and K_{ATP} current in DMEM imply that K_{ATP} channel-independent mechanism(s) may function to regulate GSIS in more physiological nutrient-rich media. Therefore, these results warrant further experimentation and re-evaluation of the role of glucose metabolism in GSIS under more physiologically relevant conditions.

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EFFECTS OF INCUBATION MEDIA ON STIMULUS-SECRETION COUPLING IN HIT-T15 CELLS: BUFFERED SALINES VERSUS NUTRIENT-RICH DMEM.

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Aims: To investigate the physiological relevance of incubation media used for β-cell metabolism and stimulus-secretion coupling.

Materials and Methods: Multinuclear NMR spectroscopy, the perforated patch-clamp method, and conventional glucose and insulin assays were used to study glucose metabolism, cellular bioenergetics, K_{ATP} current, and insulin secretion in HIT-T15 cells incubated in several traditionally used buffered salines (PBS, KRB, and HBSS) and a more physiological nutrient-rich DMEM medium. All media contained 20mM glucose (G20).

Results: We demonstrate (Table) that at elevated glucose (G20): 1) the measured glucose consumption (GCR), and insulin secretion (ISR) rates are higher in DMEM than in PBS and HBSS; 2) GCR in KRB is comparable to that measured in DMEM, but ISR is lower and similar to that measured in PBS and HBSS; 3) The K_{ATP} current is substantially lower in DMEM than in any of the salines; 4) overall activity of K_{ATP} current does not correlate to the measured GCR, ISR, the intracellular levels of ATP, and the ATP/ADP ratio. (*) Indicates statistical significance ($p<0.01$) when compared to G20-DMEM [Control]. Values are % of control (means ± SD).

	DMEM	HBSS	KRB	PBS
GCR	100±09	78±04*	115±06	76±04*
ATP	100±06	78±06*	81±03*	90±11
ATP/ADP	100±12	71±22	62±02*	74±04*
K_{ATP} Current	100	191±14*	226±70*	420±92*
ISR	100±05	31±02*	34±01*	29±01*

Conclusions: At the same glucose concentration (G20) medium composition has a prominent influence on HIT-T15 cell metabolism, electrophysiology, and insulin secretion. We believe that these observations warrant careful consideration in choosing incubation media for investigations of β-cell metabolism and stimulus-secretion coupling, and further experimentation in incubation media mimicking the in vivo environment as closely as possible.

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AN INSULINOTROPIC EFFECT OF VITAMIN D ANALOG THROUGH NON-GENOMIC SIGNAL TRANSDUCTION IN PANCREATIC β CELLS

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Aims: We investigated non-genomic effect of vitamin D via a putative membrane vitamin D receptor (mVDR) on insulin release from rat pancreatic β cells, using a specific agonist of mVDR, 1α,25(OH)2lumisterol3. **Methods:** Insulin secretory capacity by static incubation and perifusion condition from intact islets, Ca^{2+} efficacy in exocytotic system using electrically permeabilized islets, and intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) by fluorescent measurement were examined. **Results:** 1α,25(OH)2lumisterol3 (**JN**) dose-dependently augmented 16.7 mmol/l glucose-induced insulin release from rat pancreatic islets but had no effect on low concentrations (below 8.3 mmol/l) of glucose-induced insulin release. The insulinotropic effect of **JN** was also observed in the presence of a depolarizing concentration (30 mmol/l) of K^+, 200 μmol/l diazoxide and 16.7 mmol/l glucose, and in this condition, $[Ca^{2+}]i$ was increased by **JN**. These effects were completely abolished by an antagonist of mVDR, 1β,25-dihydroxyvitamin D3 (1β,25(OH)2D3), or by a blocker of voltage dependent Ca^{2+} channels (VDCC), nitrendipine. Moreover, the fact that **JN** had no effect on insulin release and elevated $[Ca^{2+}]i$ in the presence of 30 mmol/l K^+, 200 μmol/l diazoxide and 3.3 mmol/l glucose and that it had no insulinotropic effect in the presence of 5 mmol/l K^+, 200 μmol/l diazoxide and 16.7 mmol/l glucose indicates that both sufficient membrane depolarization and intracellular glucose metabolism is needed for the expression of these effects. On the other hand, **JN** did not increase Ca^{2+} efficacy in the exocytotic system. **Conclusions:** Vitamin D analog, 1α,25(OH)2lumisterol3 has a rapid insulinotropic effect through non-genomic signal transduction via mVDR , that would be dependent on the augmentation of Ca^{2+} influx through VDCC on plasma membrane, being also linked to metabolic signals derived from glucose in pancreatic β cells .

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UPTAKE, CATIONIC EFFECTS AND INSULINOTROPIC ACTION OF L-ARGININE AND ITS METHYL ESTER IN PANCREATIC ISLETS

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Aims: The stimulation of insulin release by L-arginine is currently ascribed to the transporter-mediated accumulation of this cationic amino acid inside the B-cell with resulting depolarization of the plasma membrane. In order to further evaluate such a hypothesis, the uptake, cationic and metabolic effects, and insulinotropic action of L-arginine were compared to those of its more positively charged methyl ester. **Methods:** The net uptake of L-[U-^{14}C]-arginine or its methyl ester, corrected for extracellular contamination and expressed relative to the paired ^{3}HOH space, the outflow of ^{86}Rb and ^{45}Ca from prelabelled perifused islets, the net uptake of ^{45}Ca, the oxidation of D-[U-^{14}C]glucose and utilization of D-[5-^{3}H]glucose and the release of insulin were measured in pancreatic islets isolated from fed Wistar rats. **Results:** L-arginine (10 mmol/l) increased ^{86}Rb outflow, $^{40}Ca^{2+}$ influx, ^{45}Ca net uptake and insulin release in islets exposed to D-glucose (6.0 to 16.7 mmol/l), its insulinotropic action displaying the same dependency on extracellular Ca^{2+} as that of the hexose. In contrast, its ester (also 10 mmol/l) decreased ^{86}Rb outflow, inhibited ^{45}Ca net uptake, only caused a modest increase in insulin output at intermediate concentrations of D-glucose (6.0 to 8.3 mmol/l), and even inhibited secretion at 16.7 mmol/l D-glucose. Over 5-20 min incubation, the net uptake of L-[U-^{14}C]arginine methyl ester was also much lower than that of unesterified L-[U-^{14}C]arginine. Both L-arginine and its ester failed to affect significantly D-[U-^{14}C]glucose oxidation, D-[5-^{3}H]glucose utilization or the paired ratio between these two metabolic variables. **Conclusions:** These findings are consistent with the hypothesis that the transport of L-arginine by a specific carrier system represents an essential determinant of its insulinotropic action.

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AMINO ACIDS REGULATE GLUCAGON SECRETION AND SYNTHESIS IN CLONAL ISLET ALPHA CELLS

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A relative or absolute increase in glucagon secretion from pancreatic islet α-cells is associated with every form of diabetes. However, the molecular mechanisms that regulate glucagon synthesis and secretion have not been well characterized, and may yield useful insights for therapeutic management of diabetes. Since isolation of normal islet α-cells for use in biochemical studies is difficult, in this study we have used a clonal islet α-cell line (αTC-6) as an in vitro model of α-cells. In static incubation assays, an amino acid mixture of glutamine, arginine and alanine (AA mix, 10-20 mM) induced an ~ 2-fold increase in glucagon secretion over basal levels during a one hour incubation period. The AA mix stimulation of glucagon release was attenuated by calcium channel blockade with nimodipine (1 μM). Chronic exposure of αTC-6 cells to AA mix induced a steady state elevation of glucagon mRNA levels (~ 2.5-fold increase at 24 hours). Furthermore, AA mix increased expression of a glucagon gene promoter-luciferase fusion gene in transiently transfected αTC-6 cells. The effects of AA mix on glucagon gene transcription were attenuated by nimodipine. These results demonstrate that amino acids regulate islet α-cell function with effects on both glucagon synthesis and secretion, and that these processes involve intracellular calcium signaling pathways.

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Stimulus-Secretion Coupling in Islet Cells

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ENGINEERING OF PYRUVATE- OR LACTATE-STIMULATED INSULIN SECRETION IN PANCREATIC ISLETS.

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Aims: Neither pyruvate nor lactate stimulates insulin secretion from pancreatic islets, despite their active metabolism. Recent studies demonstrated that activities of monocarboxylate transporter (MCT) and lactate dehydrogenase (LDH) are low in β-cells compared to non-β-cells, suggesting that these molecules are mainly metabolized in non-β-cells. To test whether low activities of LDH and MCT in β-cells are responsible for the lack of insulinotropic action of pyruvate or lactate in islets, we examined effects of overexpression of type 1 MCT (MCT1) and/or type A LDH (LDHA) in insulinoma INS-1 cells, or isolated rat islets. **Methods:** LDHA overexpression in INS-1 cells was achieved by stable transfection of rabbit LDHA under the tetracycline-inducible promoter. Overexpression of LDHA in islets and of MCT1 in either INS-1 cells or islets was achieved via recombinant adenovirus infection. **Results:** Inducible overexpression of LDHA resulted in an 87-fold ($p<0.001$) increase in LDH activity in INS-1 cells. Adenovirus-mediated overexpression of MCT1 increased lactate transport activity 3.4-fold ($p<0.05$) in INS-1 cells. Although overexpression of LDHA and/or MCT1 did not affect glucose-stimulated insulin secretion, LDHA overexpression caused a 2-fold ($p<0.05$) increase in lactate (2 mmol/L)-stimulated insulin secretion in INS-1 cells. Overexpression of MCT1 resulted in a 1.9-fold ($p<0.05$) increase in pyruvate (10 mmol/L) oxidation and caused pyruvate-stimulated insulin secretion (3.2-fold, $p<0.05$) in islets. Although lactate (10 mmol/L) did not stimulate insulin secretion from control and MCT1-overexpressing islets, co-overexpression of LDHA and MCT1 resulted in lactate-stimulated insulin secretion (3.5-fold, $p<0.05$) with a concomitant 3.8-fold increase ($p<0.01$) in lactate oxidation in islets. **Conclusions:** These data suggest that low activities of LDH and MCT in β-cells protect against stimulation by pyruvate and lactate, which would otherwise cause undesired insulin secretion during catabolic states, such as exercise.

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ISOMERIC AND ANOMERIC SPECIFICITY OF THE INSULINOTROPIC ACTION OF GLUCOSE PENTAACETATE

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Aims: Both the D- and L-isomers of glucose pentaacetate were recently reported to stimulate insulin release. In the present study, the anomeric stability of these esters and the anomeric specificity of their insulinotropic action were investigated. **Method:** The α- and ß-anomers of D- and L-glucose pentaacetate were synthesized by esterification of the corresponding hexoses in the presence of Lewis acid or sodium acetate, respectively. The anomeric stability of the esters in salt-balanced media was assessed over 120 min incubation at 37°C by proton NMR. Insulin release from rat islets was measured over 90 min incubation. **Results:** No anomeric interconversion and no sizeable hydrolysis of the esters of D- and L-glucose were observed after 120 min incubation at 37°C. In the presence of 2.8 mmol/l D-glucose, the anomers of D-glucose pentaacetate augmented insulin release much more efficiently than the anomers of L-glucose pentaacetate. Unexpectedly, ß-D-glucose pentaacetate increased insulin output to a greater extent than α-D-glucose pentaacetate. The two anomers of L-glucose pentaacetate yielded comparable secretory rates. At 2.8 mmol/l D-glucose, the increment in insulin output evoked by nateglinide (10 μmol/l) was also higher in the presence of ß- than α-D-glucose pentaacetate. **Conclusions:** While being consistent with the nutritional value of D- as distinct from L-glucose, these findings suggest a far-from-negligible direct contribution of the glucose pentaacetate esters themselves in their insulinotropic action, with possible preference for the C_1 configuration of ß- rather than α-D-glucose pentaacetate. This information may help in the selection of monosaccharide esters for optimization of the B-cell secretory response to hypoglycemic sulfonylureas or meglitinide analogs in NIDDM.

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EFFECTS OF EXTRACELLULAR pH ON THE INSULINOTROPIC ACTION OF α-D-GLUCOSE PENTAACETATE

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Aims: The pentaacetate ester of α-D-glucose stimulates insulin release. Its insulinotropic action appears mainly attributable to its intracellular hydrolysis, resulting in a transient and modest decrease of cytosolic pH, and further catabolism of its hexose moiety in islet cells. A direct effect of the ester itself upon a receptor apparently displaying analogy with that involved in the recognition of bitter agents by taste buds may also be operative, however. In the present study, the influence of extracellular pH on the secretory response of rat isolated pancreatic islets to α-D-glucose pentaacetate was investigated. **Materials and Methods:** Groups of 8 islets each were incubated for 90 min in the absence or presence of α-D-glucose pentaacetate (1.7 mmol/l), D-glucose (7.0 mmol/l) and L-leucine (10.0 mmol/l) at pH 7.0, 7.4 or 8.0. Changes in extracellular pH were achieved by equimolar substitution of NaCl by $NaHCO_3$. **Results:** The insulinotropic action of α-D-glucose pentaacetate was preserved, except in the absence of any other exogenous nutrient, when the extracellular pH was raised from 7.4 to 8.0. Inversely, however, when the extracellular pH was lowered to about 7.0, α-D-glucose pentaacetate inhibited both basal and D-glucose- or L-leucine-stimulated insulin output. **Conclusions:** These findings are interpreted to support a dual mode of action of α-D-glucose pentaacetate upon insulin secretion, a lowering of extracellular pH revealing a negative component of the islet B-cell functional response to such a monosaccharide ester.

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EFFECTS OF α-D-GLUCOSE PENTAACETATE, ß-L-GLUCOSE PENTAACETATE AND ß-D-GALACTOSE PENTAACETATE ON THE REDOX STATE OF PURIFIED RAT ISLET B-CELLS

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Aims: In order to assess the nutritional value of α-D-glucose pentaacetate (α-D-GluPA), ß-L-glucose pentaacetate (ß-L-gluPA) and ß-D-galactose pentaacetate (each 1.7 mmol/l), their effects on NAD(P)H autofluorescence was measured in rat islet B-cells deprived of any other exogenous nutrient or exposed to either 8.3 mmol/l D-glucose or 10.0 mmol/l L-leucine. **Methods:** Dispersed rat islet cells loaded with Fluo3-AM were analyzed on a flow cytometer to measure their NAD(P)H autofluorescence. The cytometer was calibrated with multi-level fluorescent beads immediately before each series of measurements. **Results:** Within 1 min, D-glucose, but not L-leucine, increased the NAD(P)H signal. Except in the presence of D-glucose, it was further increased by α-D-gluPA. Such was not the case with the other two esters. A comparable situation was found after 45 min incubation, except that ß-L-gluPA now also slightly but significantly increased the NAD(P)H signal in cells exposed to L-leucine. The paired ratio between the 45th min and initial readings was always higher than unity in cells exposed to D-glucose, whilst being lower than unity in cells deprived of any exogenous nutrient. Relative to the latter value, it was increased by α-D-gluPA, but not the two other esters. In the presence of L-leucine, both α-D- and ß-L-gluPA also increased this paired ratio above the corresponding control value recorded in the sole presence of the amino acid. **Conclusions:** These findings are consistent with the view that, among the 3 esters, only α-D-gluPA acts as a nutrient in B-cells. The late increase of the NAD(P)H signal in the cells exposed to both L-leucine and ß-LgluPA may be attributable to both the modest nutritional value of the amino acid and its enhancement by ß-L-gluPA secondary to mitochondrial Ca^{2+} accumulation.

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EXPRESSION OF THE α-GUSTDUCIN, A TASTE-CELL-SPECIFIC G PROTEIN, IN ISLET B-CELLS

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Aims: It was recently reported that selected esters of non-metabolized monosaccharides, such as α- or ß-L-glucose pentaacetate, stimulate insulin release both *in vitro* and *in vivo*. The insulinotropic action of such esters cannot be attributed to the catabolism of either their glucidic or acidic moieties. It was proposed, therefore, that L-glucose pentaacetate itself may directly interact with a yet unidentified receptor, in a manner reminiscent of that involved in the recognition of bitter compounds by taste buds. These esters indeed display a bitter taste. Since the taste of bitter compounds is thought to be transduced at the intervention of a taste-specific-cell G protein, gustducin, it was now investigated whether α-gustducin is expressed in pancreatic islet cells. **Methods:** Specific primers designed according to the published sequence of the rat lingual epithelium were used in rt-PCR amplification to obtain a specific fragment of the G protein α subunit. After subcloning and sequencing, the PCR products were labelled by the digoxigenin method and used as specific probe in order to determine the expression of the α-gustducin by Northern hybridization. **Results:** After rt-PCR amplification, a specific fragment of the α-gustducin was obtained in rat tongue (used as positive control), pancreatic islets and pancreatic purified B-cells. The sequence of the α-gustducin fragment obtained from purified B-cells was comparable to that reported for the rat lingual epithelium. Specific transcripts of the α-gustducin gene were identified by Northern blot of total RNA from rat lingual epithelium, pancreatic islets and RINm5F cells. **Conclusions:** These results demonstrate the expression of the taste-cell-specific G protein α-gustducin in pancreatic islet B-cells. Together with the discovery that L-glucose pentaacetate stimulates insulin, this could open the way to identification of a novel pathway for activation of islet B-cells.

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METABOLIC AND SECRETORY INTERACTIONS BETWEEN D-GLUCOSE AND D-FRUCTOSE IN ISLETS FROM GK RATS

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Aim: It was recently proposed that the reciprocal effects of D-glucose and D-fructose on their respective metabolism in islets from normal rats may optimize the insulin secretory response to these hexoses after food intake. The present study aims at investigating whether a comparable situation prevails in islets from hereditarily diabetic GK rats. **Methods:** Metabolic variables and insulin release were measured over 120 min incubation of isolated islets in the presence of D-glucose and/or D-fructose (10 mmol/l each). **Results:** In GK, like in normal rats, D-fructose failed to affect significantly the utilization of D-[5-^{3}H]glucose and oxidation of D-[U-^{14}C] glucose, D-[1-^{14}C]glucose or D-[6-^{14}C]glucose. Likewise, in GK as in normal animals, D-glucose inhibited D-[5-^{3}H]fructose utilization, but augmented D-[U-^{14}C]fructose oxidation. The concentration-response relationship for such an effect was also comparable in control and GK rats. Despite such analogies between normal and GK rats, D-fructose was less efficient in the latter than former animals in its capacity to augment glucose-stimulated insulin release. Thus, the concentration of D-fructose required to cause a significant increase in insulin output from islets exposed to D-glucose amounted to 30 mmol/l in GK rats, as distinct from 10 mmol/l or less in control rats. Moreover, the relative extent of such an increase, at 30 mmol/l D-fructose, was lower in GK than control rats. **Conclusions:** The contrast between a normal metabolic and altered secretory response to D-fructose in GK rats reinforces the view that the insulinotropic action of the ketohexose cannot be entirely accounted for by its nutritional value in islets cells.

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INTRACELLULAR SIGNALLING AND 4-HYDROXYISOLEUCINE INSULINOTROPIC EFFECT.

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We have previously reported that 4-hydroxyisoleucine (4-OH-Ile), an amino acid extracted and purified from fenugreek seeds (*Trigonella foenum graecum L*) stimulates insulin release only in the presence of intermediate to high glucose concentrations and does not interact with other insulinotropic agents as leucine or arginine. Moreover, we have shown that 4-OH-Ile has only a weak effect on ATP dependent potassium conductance. The present study was aimed at investigating further the pancreatic B cell mechanisms involved in the stimulus-secretion coupling of 4-OH-Ile. For this purpose, in islets of Langerhans isolated from normal rats and incubated in the presence of intermediate glucose concentration (8.3 mmol/l) different sets of experiments were performed. Our results show that: (i) the addition of a calcium channel blocker, verapamil (50 µmol/l), prevents the insulinotropic effect of 4-OH-Ile (200 µmol/l); (ii) 4-OH-Ile (200 µmol/l) increases glucose induced insulin release (2.02 ± 0.18 ng/islet/30 min versus 1.58 ± 0.12 for control, $p < 0.05$) without modifying cAMP content (1.2 ± 0.1 fmol/islet versus 1.1 ± 0.05 for control and 12.3 ± 1.2 for 0.5 mmol/l IBMX); (iii) glucose utilization was significantly enhanced in the presence of 4-OH-Ile at 200 µmol/l (70.2 ± 9.1 pmol H_2O/islet/90 min versus 45.1 ± 4.3 for control, $p < 0.05$), without any significant change in glucose oxydation (11.0 ± 1.7 pmol CO_2/islet/90 min versus 13.0 ± 1.8 for control) nor in lactate formation (1.11 ± 0.09 µmol/islet/90 min versus 0.99 ± 0.16 for control and 2.03 ± 0.05 for 1 mmol/l phenformin). In conclusion, our data show that calcium influx is necessary to the insulinotropic effect of 4-OH-Ile; this effect is not related to an increase in cAMP content, but appears rather mediated by an action on early steps of glucose metabolism.

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PARTICIPATION OF FRUCTOKINASE TO D-FRUCTOSE PHOSPHORYLATION IN PANCREATIC ISLETS

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Aims: In islet homogenates, D-fructose phosphorylation is catalyzed by hexokinase, glucokinase and fructokinase. The respective contributions of each of these enzymes to D-fructose phosphorylation in intact islets, incubated in the absence or presence of D-glucose, is not known, however. To progress on such an issue, the metabolism of D-glucose and/or D-fructose was investigated, therefore, in intact islets. **Methods:** Isolated pancreatic islets from normal rats, GK rats and adult animals injected with streptozotocin during the neonatal period (STZ rats) were incubated for 120 min for measurements of $^{14}CO_2$ and ^{3}HOH production from [5-^{3}H]- and [U-^{14}C]-labelled D-glucose and/or D-fructose (10 mmol/l each). **Results:** D-fructose failed to affect significantly the metabolism of D-glucose. D-glucose, however, lowered D-[5-^{3}H]fructose utilization, whilst increasing D-[U-^{14}C]-fructose oxidation. The most salient finding consisted in the fact that, in the concomitant presence of both hexoses, the paired ratio between D-[U-^{14}C]-fructose oxidation and D-[5-^{3}H]fructose utilization (49.4 ± 2.2 %; n = 53) was higher than that between D-[U-^{14}C]glucose oxidation and D-[5-^{3}H]-glucose utilization (38.5 ± 1.1 %; n = 40). From these ratios, and assuming isotopic equilibration of D-[U-^{14}C]glucose 6-phosphate and D-[U-^{14}C]-fructose 6-phosphate, it was calculated that the fractional contribution of fructokinase to the phosphorylation of D-fructose accounted, in control and GK rats, to at least 22.0 ± 5.4 %. Such a percentage was even higher in islets from STZ rats. **Conclusions:** The present findings indicate that fructokinase participates to a far-from-negligible extent to the phosphorylation of D-fructose in intact islets. They also imply that the formation of ^{3}HOH from D-[5-^{3}H]fructose underestimates sensibly the true phosphorylation rate of the ketohexose.

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Potassium Channels in the Regulation of Insulin Secretion

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DOWNREGULATION OF K_{ATP}-CHANNEL-DEPENDENT AND -INDEPENDENT INSULINOTROPIC ACTIONS OF TOLBUTAMIDE

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K_{ATP}-channel-dependent and K_{ATP}-channel-independent insulin-releasing actions of tolbutamide, were examined in the clonal BRIN-BD11 cell line. In acute 20 min incubations (n=6), 50-400 µmol/l tolbutamide stimulated a 1.3-2.1-fold increase ($p<0.01$-$p<0.001$) in insulin release at 1.1 mmol/l glucose (2.02 ± 0.18 ng/10^6 cells/20 min, mean ± SEM). Raising the glucose concentration to 16.7 mmol/l in the presence of depolarizing concentrations (200-400 µmol/l) of tolbutamide stimulated a 1.4-fold ($p<0.01$) increase in insulin secretion, reinforcing the K_{ATP}-channel-independent actions of glucose. Under depolarizing conditions (30 mmol/l KCl and 16.7 mmol/l glucose; 14.37 ± 0.51 ng/10^6 cells/20 min) 100-400 µmol/l tolbutamide evoked a 1.2-1.3-fold ($p<0.05$-$p<0.01$) dose-dependent insulin secretory response. Culture (3-18 h) with 100 µmol/l tolbutamide reduced (16-31%, $p<0.01$) subsequent responsiveness to acute challenge with 200 µmol/l tolbutamide, in a time-dependent manner ($p<0.01$). Similarly, 18 h culture with 100 µmol/l tolbutamide removed the K_{ATP}-channel-independent actions of this agent without significantly reducing the depolarizing effect of 30 mmol/l KCl itself. Notably, culture with tolbutamide also caused a 20% decrease ($p<0.001$) in the insulin secretory response to 25 µmol/l forskolin (7.55 ± 0.15 ng/10^6 cells/20 min) but not that of 10 nmol/l phorbol-12-myristate 13-acetate. These data demonstrate that prolonged exposure to tolbutamide downregulates both the K_{ATP}-channel-dependent and -independent insulin-secretory actions of this antidiabetic drug, indicating synergistic pathways mediated by common sulphonylurea binding site(s).

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DESENSITIZATION OF INSULIN SECRETION BY INHIBITORS OF ATP-DEPENDENT K^+ CHANNELS

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Aims: to define how prolonged stimulation of pancreatic islets by inhibitors of K_{ATP} channels leads to a decrease of the secretory responsiveness. Incubation of isolated mouse pancreatic islets in a high concentration of the sulfonylurea, tolbutamide (500 µM in RPMI 1640 with 5 mM glucose for 18 h) virtually abolished the insulinotropic effect of this compound upon renewed stimulation, while control-incubated islets showed a prompt and robust secretory response. A homologous desensitization of similar magnitude occured after incubation in high concentrations of imidazolines (100 µM phentolamine or 100 µM alinidine), while the one induced by quinine (100 µM), was less marked. The insulin content of islets desensitized by exposure to the above compounds was not significantly reduced as compared with control-incubated islets. When islets were incubated under a strongly depolarizing condition (40 mM K^+ for 18 h), the secretory response of such islets to subsequent stimulation with tolbutamide, phentolamine, alinidine or quinine was reduced, but not to the same extent as by homologous desensitization. The increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) by phentolamine and alinidine was suppressed in desensitized B-cells, while the $[Ca^{2+}]_i$ increase by reexposure to quinine was partially reduced and that by tolbutamide unchanged in comparison to control-incubated B-cells. K_{ATP} channels in intact B-cells were blocked and the membrane potential was reduced after culture in the presence of phentolamine, alinidine or quinine, but not tolbutamide. Conclusion: the desensitization of insulin secretion induced by inhibitors of K_{ATP} channels is not due an unresponsiveness of K_{ATP} channels, rather, insulin secretion appears to be inhibited at later stages, e.g. by a block of Ca^{2+} influx as induced by phentolamine, or at steps distal to Ca^{2+}-influx as induced by tolbutamide.

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K_{ATP} CHANNEL ACTIVATION BY NICORANDIL AND RILMAKALIM

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Aims: Potassium channel openers (KCOs) comprise a structurally diverse group of drugs with a broad spectrum of potential therapeutic applications (e.g. hypoglycemia, hypertension). These drugs exert their effects on excitable cells (e.g. pancreatic β-cells, vascular smooth muscle and cardiac myocytes) by opening ATP-sensitive potassium channels (K_{ATP} channels) thus shifting the membrane potential towards the reversal potential for potassium and reducing cellular electrical activity. K_{ATP} channels are composed of a small inwardly rectifying K^+ channel subunit ($K_{IR}6.x$) plus a sulfonylurea receptor (SUR1, SUR2A or B). Recently, it has been established that SURs are the KCO receptors of K_{ATP} channels and KCOs of various chemical classes (pinacidil, levcromakalim, diazoxide) were shown to induce monophasic displacement curves suggesting binding to a single class of receptor sites. However, nicorandil and rilmakalim have previously been proposed to bind to receptor sites distinct from the pinacidil-type. To test this assumption, binding of these drugs and effects on recombinant SUR2B/K_{IR}6.2 channels were analyzed. **Methods:** Following transient expression of SUR2A or B in COS-7 cells, specific binding of nicorandil and rilmakalim was assessed by displacement studies using [^{3}H]P1075. Potencies to activate recombinant SUR2B/K_{IR}6.2 channels were measured using the inside-out configuration of the patch-clamp technique. **Results:** [^{3}H]P1075 competition experiments revealed monophasic displacement curves with Hill coefficients close to one and dissociation constants for binding of nicorandil and rilmakalim to SUR2B of 9 ± 0.6 µM and 30 ± 3 nM, respectively (N = 5 each). Potencies for activation of SUR2B/K_{IR}6.2 channels were 6.8 or 7.5-fold lower than binding affinities with Hill coefficients for the concentration-activation curves significantly higher than one (1.29 or 1.54, respectively). Affinities for binding to SUR2A were 2.5 to 4-fold lower than that for SUR2B. **Conclusion: Nicorandil and rilmakalim exert their effects on K_{ATP} channels by interaction with the pinacidil-type KCO site on sulfonylurea receptors.**

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HYDROCHLOROTHIAZIDE: AN HYPERGLYCAEMIA-INDUCING AGENT AND K_{ATP} CHANNEL AGONIST IN HUMAN ß-CELLS AND CLONAL INSULIN-SECRETING CELLS.

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Aims: Thiazide diuretics are widely used to treat hypertension but are also known to impair glucose tolerance and even induce diabetes in some patients. Hydrochlorothiazide (HCT) is one such diuretic with hyperglycaemic effects. Here, we examined whether the diabetogenic actions of HCT involve inhibition of insulin secretion and the modulation of ATP-sensitive potassium (K_{ATP}) channels in insulin-secreting cells. **Methods:** Patch clamp experiments were used to monitor the regulation of K_{ATP} channels in isolated human ß-cells and in the glucose-responsive ß-cell line, BRIN-BD11. Insulin secretion was assessed by RIA. **Results:** In BRIN-BD11 ß-cells, HCT (1-100µM) inhibited both 15mM glucose- (n=8) and 100µM tolbutamide (Tol.)-induced secretion (n=8). Significantly, whilst 1µM HCT inhibited insulin release to near basal values (0.82±0.03ng/10^6 cells/20 min; 100%) under both conditions, the K_{ATP} channel agonist diazoxide (DZ; 1µM) was without effect: glucose (Glu.) = 173±17%, Glu. + HCT = 103±10%, Glu. + DZ = 175±21% (n=8); Tol. = 169±8%, Tol. + HCT = 120±10%, Tol. + DZ = 167±16% (n=8). At higher concentrations, both compounds were similarly effective at inhibition of insulin release. Using the inside-out configuration of the patch-clamp technique, HCT (1µM-100µM, n=12-38) activated K_{ATP} channels and reversed 0.5mM ATP-induced channel inhibition. In the presence of ATP, 1µM HCT increased K_{ATP} channel activity by 2.3±0.3 fold (n=9) and similar effects were seen with 100µM HCT (3.5±1, n=6) and 50µM HCT (3.1±0.4, n=5). In isolated human ß-cells (n=17), 1µM HCT caused a 2.3±0.3 fold (n=6) increase in K_{ATP} channel activity and 100µM HCT activated ATP-inhibited channels by 1.98±0.2 fold (n=6). Finally, in both BRIN-BD11 and human ß-cells, HCT was consistently found to be a more potent agonist of K_{ATP} channels than DZ (n=8). **Conclusions:** these data show that the thiazide diuretic HCT is an agonist of K_{ATP} channels in ß-cells, and that this is causally related to the inhibition of insulin release.

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THE IMIDAZOLINE EFAROXAN INFLUENCES SUR1 PROTEIN EXPRESSION IN INSULIN-SECRETING CELLS.

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Aims: Imidazoline compounds, such as phentolamine and efaroxan (EFX) are insulin secretagogues. Here, we have examined the effects of imidazoline receptor down-regulation on insulin-secreting cell function using the glucose-responsive ß-cell line, BRIN-BD11. **Methods:** Patch clamp experiments were used to monitor the regulation of K_{ATP} channels, microfluorimetry with fura-2 was used to study $[Ca^{2+}]_i$ homeostasis, insulin secretion was measured by RIA and SUR1 protein expression was assessed by Western immunoblotting using a specific SUR1 receptor antibody. **Results:** Pre-exposure of BRIN-BD11 ß-cells for 20 hours to 50µM EFX selectively inhibited EFX (200µM)-induced insulin release, but not the responses of cells to glucose (15mM), tolbutamide (200µM) or a depolarising concentration of KCl (25mM) (n=6). When pre-incubated with 50 to 500µM EFX, there was a consistent increase in SUR1 protein abundance at all concentrations tested (n=4), but this did not prevent EFX (200µM, n=13)- or KCl- (50mM, n=11) induced increases in $[Ca^{2+}]_i$ in the same batches of cells. The density and the regulation of K_{ATP} channels in the same groups of cells were also investigated. Using the patch-clamp technique we found K_{ATP} channels were operational in intact cells (n=6) and were modulated by intracellularly applied nucleotides (n=14), and imidazolines (n=7). Furthermore, whilst there was no significant difference in the density of channels in cells pre-exposed to either 50µM EFX or 200µM EFX, there was a significant increase in channel numbers in cells pre-treated with 500µM EFX, and this correlated well with a more marked increase in SUR1 protein expression at this concentration: peak current values; control=28.2±4pA, 50µM EFX=28.1± 12pA, 200µM EFX=26.4±4pA and 500µM EFX=94.7±39pA, n=3-20. **Conclusions:** these data show that pre-exposure to EFX selectively down-regulates EFX-induced insulin release at concentrations that do not affect $[Ca^{2+}]_i$ signalling or K_{ATP} channel function despite an increase in SUR1 protein abundance.

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STIMULATION OF INSULIN SECRETION FROM ISOLATED HUMAN PANCREATIC ISLETS BY THE β-CARBOLINE, HARMANE.

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Aims: Recent studies have revealed that pancreatic β-cells are equipped with a binding site for imidazoline ligands which is coupled to K_{ATP} channels and promotes a rise in insulin secretion. Thus, the site may represent an appropriate target for the development of new insulin secretagogues for use in the management of type 2 diabetes. Although the functional pharmacology of the islet imidazoline receptor has been defined, the precise structure-activity relationships for agonism are not known. **Methods**: We have screened a range of molecules, of different structural classes, in an attempt to identify new ligands for this site which display insulin secretagogue activity in isolated human islets. **Results:** One such molecule, harmane (a β-carboline derivative) was found to stimulate insulin secretion from human islets incubated in the presence of 6mM glucose (6mM glucose: 2.1±0.2 ng/islet/h; 6mM glucose + 100µM harmane: 3.4±0.4 ng/islet/h; $p<0.01$). In common with imidazoline ligands, harmane also completely prevented the inhibition of glucose-induced insulin secretion mediated by the K_{ATP} channel agonist diazoxide (20mM glucose + 250µM diazoxide: 1.0±0.06 ng/islet/h; 20mM glucose + diazoxide + 100µM harmane: 3.4±0.6 ng/islet/h; $p<0.001$). This effect was dose-dependent over the range 0.1-100µM harmane (EC50 ~ 5-10µM) and was prevented by inclusion of the imidazoline antagonist KU14R (20mM glucose + 200µM diazoxide: 0.6±0.06 ng/islet/h; 20mM glucose + diazoxide + 100µM harmane: 2.6±0.25 ng/islet/h; 20mM glucose + diazoxide + harmane + 100µM KU14R: 0.7±0.1 ng/islet/h' $P<0.001$). **Conclusions:** These results reveal that harmane can stimulate insulin secretion and suggest that it may interact with imidazoline binding sites associated with K_{ATP} channels. This raises the possibility that β-carbolines may be a useful class of molecules for development of novel insulin secretagogues.

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GENERATION OF ANTI-IDIOTYPIC ANTI-RECEPTOR ANTIBODIES AGAINST THE PANCREATIC ISLET IMIDAZOLINE RECEPTOR

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Aims: The imidazoline receptor which mediates the action of imidazoline insulin secretagogues such as efaroxan has, as yet, eluded complete characterisation. The approach that we have taken to the isolation and purification of the imidazoline receptor is the development of anti-idiotypic anti-receptor antibodies. **Methods:** Firstly, polyclonal anti-ligand antibodies were generated in Dutch rabbits using efaroxan (coupled to BSA) as immunogen. Following purification on an efaroxan-affinity column, the antibodies were used to immunise a second set of Dutch rabbits. Anti-idiotypic antibodies were detected using an ELISA, with membranes from the clonal cell line RINm5F as the coating antigen. **Results:** In Western blot analysis, the antisera recognised four highly-immunoreactive bands in membranes from RINm5F cells, with a molecular size range of 45 - 90kDa. This immunoreactivity profile appears to be islet B-cell specific since these same four bands were observed in other clonal B-cell lines (NES, HIT-T15, βTC), but were totally absent in membranes of islet non B-cell lines, including RINT3, and in exocrine pancreas. In membranes from islets from rat and human, the antisera recognised only one band of 55kDa, which corresponded to one of the 4 bands observed in the clonal B-cells. Isoelectric focussing of solubilised RIN cell membranes revealed that the immunoreactive bands are acidic peptides, and electrophoresis under non-reducing conditions indicated that the tight association of these peptides are not via disulphide interactions. Immunofluoresecence staining of HIT cells grown on coverslips and of human pancreatic sections revealed immuno-localisation in the plasma membranes of B-cells, with granular fluorescence present in some cells, suggesting localisation in secretory granules. **Conclusions:** The polyclonal anti-idiotypic antibodies appear to recognise islet B-cell-specific targets and thus, may be useful tools for the isolation of islet imidazoline receptor involved in insulin release.

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DOES THE K_{ATP} CHANNEL PARTICIPATE IN THE REGULATION OF GLUCAGON SECRETION?

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Glucagon release from the α-cells is inversely dependent of the blood glucose level but the cellular mechanisms involved are unknown. The aim of this study was to investigate, using the patch-clamp technique, if the α-cell is equipped with ATP-dependent (K_{ATP}) K^+ channels and if they play a role in the regulation of glucagon secretion. Single rat α-cells generate action potentials in the absence of glucose, which are inhibited by the addition of 20 mM of the sugar. The electrical activity inhibited by glucose could be restored by the application of the sulphonylurea (SU) tolbutamide thus indicating the involvement of a SU receptor. In whole-cell experiments, the wash-in of a pipette solution containing a mixture of 0.3 mM ATP and 0.3 mM ADP activated a K^+ current which could be blocked by tolbutamide (K_i= 6 µM). Furthermore, the K_{ATP} channel opener diazoxide, but not pinacidil or cromakalim, activated a whole-cell K^+ conductance and inhibited α-cell electrical activity. In inside-out patches, we observed a K^+ channel with a single channel conductance of 70 pS in symmetrical (high) K^+. This channel was inhibited by intracellular ATP (K_i=17 µM). Channel activity in the presence of ATP (50 µM) was increased >4-fold by the inositol phosphate lipid PIP_2 (5 µM). The K_{ATP} channel was activated by diazoxide, ADP and GDP whereas GTP or UTP had no effect on its open probability. Finally, in-situ hybridisation revealed that cells staining positive for glucagon also express SUR1 and Kir6.2 but *not* SUR2 or Kir6.1. In conclusion, the α-cell possess K_{ATP} channels with biophysical properties expected for a SUR1/Kir6.2 channel complex. The precise role of this channel in the regulation of glucagon release remains to be determined.

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DETECTION OF KvLQT1 CHANNELS IN INS-1 CELLS AND ITS ROLE IN INSULIN SECRETION

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KvLQT1 is found in excitable (heart) and non-excitable epithelial cells. KvLQT1 associates together with Isk (Mink) forming small K^+-channels (IK_s). Upon depolarisation to positive potentials IK_s current slowly activates reaching maximum only after several seconds. The aim of the present study was to analyse whether KvLQT1 and Isk are present in INS-1 cells and to examine whether the IK_s channel contributes to the regulation of insulin secretion. Voltage steps from −80 mV to 50 mV lead to an activation of an outward whole cell current of 0.11 ± 0.01 nA/pF at 22°C and 0.33 ± 0.02 nA/pF at 34°C (n=18). This current is affected by the chromanol HOE 293B, a specific inhibitor of IK_s, in a concentration dependent manner (5 µM inhibited 3.3 ± 0.7 % (n=3) of the outward current, 10 µM 7.9 ± 0.7 % (n=3), 20 µM 15.6 ± 0.5 % (n=3), 50 µM 23.6 ± 2.32 % (n=5) and 100 µM 42.6 ± 5.5 % (n=7)) at room temperature. Similar results have been obtained at 37°C. Since maximal activation of 293B sensitive current was completed in less than 50 ms, the channel properties in insulin secreting cells do not correspond to the heart IK_s channel but to a channel which may only comprise KvLQT1. Using RT-PCR we found KvLQT1- mRNA in INS-1 cells. In the presence of tolbutamide (100 µM) 293B increased insulin secretion 3.4-fold as assessed after 30 min incubation of the cells. 293B had no significant effect on basal secretion (0.5 mM glucose) nor on secretion elevated by forskolin (5 µM) or glucose (16.7 mM). Adrenaline still inhibited secretion in the presence of the chromanol. In conclusion, it is suggested, that KvLQT1 is functionally expressed in INS-1 cells and it might contribute to repolarisation in stimulated cells.

PS 24
Ca^{2+} Signalling in β-Cells

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THE ENDOPLASMIC RETICULUM CONTRIBUTE TO OSCILLATIONS OF CYTOSOLIC CA^{2+} TRIGGERED BY PERIODIC CA^{2+} INFLUX IN B-CELLS.

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In pancreatic B-cell, glucose induces oscillations of the free cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) that mainly result from periodic influx of Ca^{2+}. Aim: The role of the endoplasmic reticulum (RE) in these oscillations is controversial and was investigated here. Materials and Methods: $[Ca^{2+}]_c$ (fura-2) and membrane potential (intracellular microelectrode) were measured on whole islets or single B-cells from mice. Results: $[Ca^{2+}]_c$ oscillations occurring in response to glucose or artificially induced by pulses of high K^+ (in the presence of diazoxide) displayed a descending phase with two components: a fast drop due to the closure of voltage-dependent Ca^{2+} channels followed by a much slower decrease independent of Ca^{2+} influx. Thapsigargin (TG) or cyclopiazonic acid (CPA), specific blockers of the SERCA pump, increased the amplitude of $[Ca^{2+}]_c$ oscillations suggesting that the ER buffers the rise in $[Ca^{2+}]_c$ by rapidly taking up Ca^{2+} during the upstroke of $[Ca^{2+}]_c$ oscillations. They also suppressed the slow component of the descending phase of $[Ca^{2+}]_c$ oscillations, which demonstrates that this phase reflects Ca^{2+} release from the ER. The slow recovery Ca^{2+} phase did not involve depolarization-, Ca^{2+}- or IP_3-induced calcium release, and could be reproduced at the end of a rapid rise in $[Ca^{2+}]_c$ triggered from caged Ca^{2+}. On the other hand, TG depolarized the plasma membrane of islets, suggesting that the filling state in Ca^{2+} of the ER modulates the membrane potential. Conclusions: These observations suggest that the Ca^{2+} concentration in the ER ($[Ca^{2+}]_{ER}$) oscillates in parallel with $[Ca^{2+}]_c$ oscillations. Ca^{2+} release from the ER during these oscillations might rythmically depolarize the plasma membrane and, thereby, modulate the bursting behaviour of B-cells, and eventually $[Ca^{2+}]_c$ oscillations themselves during glucose stimulation.

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THE REGULATION OF CYTOSOLIC Ca^{2+} SIGNALS IN A HUMAN ß-CELL LINE, NES 2Y.

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Aims; NES 2Y cells are a human insulin-secreting cell line derived from a patient with persistent hyperinsulinaemic hypoglycaemia of infancy (PHHI). PHHI ß-cells lack operational K_{ATP} channels and constitutively secrete insulin under basal conditions. Here, we examined the control of intracellular Ca^{2+} homeostasis ($[Ca^{2+}]_i$) in this novel ß-cell line. **Methods;** $[Ca^{2+}]_i$ changes were monitored using fura-2 microfluorimetry, and the properties of voltage-gated Ca^{2+} channels examined by patch-clamp techniques. **Results;** Like acutely isolated PHHI ß-cells, NES 2Y cells have basal rates of insulin release that are elevated under non-stimulatory conditions; ~ 0.9±0.1ng/10^6 cells/hr (n=4) *vs.* ~ 0.1±0.05ng /10^6 cells/hr in MIN 6 ß-cells (n=4). When NES2Y cells were challenged with glucose (20mM; n=17), tolbutamide (0.1-0.2mM; n=24) or KCl (40mM; n=23) each agent failed to elevate $[Ca^{2+}]_i$, and these data are explained by the functional loss of both K_{ATP} channels (n=19) and voltage-gated Ca^{2+} channels (n=10). Release of Ca^{2+} from intracellular stores was studied in NES2Y cells using a combination of ATP and acetylcholine (100µM each). In 50% of experiments this produced an oscillatory rise in $[Ca^{2+}]_i$ and the average peak response increased $[Ca^{2+}]_i$ by 174±42 nM (n=18/21). Similar experiments in control human islets produced monophasic rises of 56±4 nM (n=74/78). Acute removal of extracellular Ca^{2+} ($[Ca^{2+}]_o$) gave only a modest fall in $[Ca^{2+}]_i$ (-21nM in 1/5 cells), whereas raising $[Ca^{2+}]_o$ to 10mM produced a more marked rise when compared to human controls: 402±120nM (n=7) *vs.* 46±6 (n=35/41). Together, these data suggest that $[Ca^{2+}]_i$ homeostasis in NES2Y ß-cells is dysregulated and that non-voltage-dependent Ca^{2+} entry may play a greater role in governing insulin release than in controls. Significantly, maitotoxin an activator of non-selective cation channels, evoked a larger rise in $[Ca^{2+}]_i$ in NES2Y ß-cells than in human controls: 765±99nM (1nM; n=10) *vs.* 210±40nM (10nM n=4). **Conclusions**; the human ß-cell line NES 2Y is a functional model of the PHHI ß-cell, and is a convenient system to study "K_{ATP} channel-independent pathways" of insulin release.

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β-CELL RECRUITEMENT BY GLUCOSE EVIDENCED BY MEASURING CYTOSOLIC CA^{2+} IN ISLET CELL CLUSTERS

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Individual β cells are functionally heterogeneous as shown by their variable glucose-dependence for insulin biosynthesis and secretion. Glucose-induced changes in $[Ca^{2+}]_i$, a key step in the stimulation of insulin secretion, are also heterogeneous in single β cells. However, within the islet $[Ca^{2+}]_i$ oscillations are well synchronized probably because of β cell coupling. In this study, we used clusters of 6-15 mouse islet cells to evaluate whether β cell recruitment occurs before or after generation of the Ca^{2+} signal. Clusters cultured for 1 or 2 days in RPMI medium (10 mmol/l glucose) were loaded with fura 2, and $[Ca^{2+}]_i$ was recorded with an imaging system during stepwise increases in the glucose concentration (6, 7, 8, 10 mmol/l). The proportion of clusters showing a $[Ca^{2+}]_i$ rise increased from 30 to 90% (day 1) and from 45 to 100% (day 2) between 6 and 10 mmol/l glucose, indicating that the threshold sensitivity to glucose differs between clusters. Within responsive clusters, 70-80% of the cells were active at 6 mmol/l and 95-100% at 8-10 mmol/l glucose. This activity consisted in fast and slow $[Ca^{2+}]_i$ oscillations that were synchronous between cells. In clusters responding homogeneously already at 6 mmol/l glucose, the magnitude of $[Ca^{2+}]_i$ changes increased with the glucose concentration, which reflects modulation of the individual cell response. In conclusion, β cell recruitment by glucose can occur at the stage of the $[Ca^{2+}]_i$ response. However, this type of recruitment is limited to a narrow range of glucose concentrations probably because of cell electrical coupling. Modulation of the action of Ca^{2+} on exocytosis may be more important for recruitment of secreting β cells.

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CARBACHOL-INDUCED CA^{2+}-SIGNALING AND GLUCOSE METABOLISM IN MOUSE BETA-CELLS

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Aims: The regulation of insulin secretion is multifactorial involving nutrients like glucose (glc) and neurohumeral factors like acetylcholine (ACH), that activate the Ca^{2+}/phospholipase C (PLC) signaling pathway. The actions of ACH on the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) are glc-dependent. As a rise in ATP is a critical signaling event in glc-dependent regulation of B-cell functions, we characterized the metabolic steps by which glc exerts its synergistic effects on ACH-linked Ca^{2+}-signals. **Methods:** $[Ca^{2+}]_i$ was measured in single fura-2 loaded mouse B-cells obtained from female NMRI mice and cultured for 2-3 days in RPMI medium. **Results:** In the presence of glc (6 mM) the ACH-analogon carbachol (3 µM) increased $[Ca^{2+}]_i$ by 327 ± 47 nM and 68 ± 14 nM at its peak or plateau, respectively (n=18). In glc (0 mM) the carbachol (3 µM)-induced increase in $[Ca^{2+}]_i$ amounted to 12 ± 5 % (n=11) of the increase in glc (6 mM). In glc (6 mM) sodium arsenate (2 mM), which prevents net glycolytic production of ATP without inhibiting glycolysis, had no significant effect on the carbachol (3 µM)-induced Ca^{2+}-signal. By contrast, the mitochondrial pyruvate transport inhibitor 2α-methoxycyanocinnamate (1 mM) inhibited the carbachol (3 µM)-induced increase in $[Ca^{2+}]_i$ by 80 ± 10 % of controls (n = 8). Rotenone (1 µM) and antimycin A (50 nM) which inhibit complex 1 and 2 of the mitochondrial respiratory chain, inhibited the carbachol (3 µM)-induced increase in $[Ca^{2+}]_i$ by 90 ± 8 % (n = 7) or 97 ± 3 % (n = 4) of controls. **Conclusions:** ATP generated from mitochondrial oxidative glucose metabolism underlies the synergisic effect of the extracellular metabolic glc-signal on ACH-linked Ca^{2-}-signaling in mouse B-cells. Supported by DFG grant Scho 466/1-3.

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TOLBUTAMIDE INDUCES TETRODOTOXIN-RESISTANT OSCILLATIONS OF CYTOPLASMIC Na^+ IN PANCREATIC β-CELLS

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Aims: Previous studies have demonstrated that both glucose and sulfonylureas promote the entry of Na^+ in pancreatic β-cells. It was therefore analyzed if tolbutamide mimics glucose in inducing oscillations of the cytoplasmic concentration of Na^+ ($[Na^+]_i$). Materials and Methods: $[Na^+]_i$ was measured in mouse ß-cells using dual wave-length microfluorometry and the indicator SBFI. Results: The addition of 0.1 or 1.0 mmol/l tolbutamide to a medium containing 3 mmol/l glucose and 1.3 mmol/l Ca^{2+} resulted in elevation of $[Na^+]_i$, which in some of the β-cells (15%) was manifested as oscillations (frequency 0.08/min; amplitude 4.6 mmol/l). The analysis of the tolbutamide-induced $[Na^+]_i$ oscillations was facilitated by replacing extracellular Ca^{2+} with 5 mmol/l Sr^{2+}. In this case about 50 % of the cells oscillated in response to 0.1 or 1.0 mmol/l tolbutamide. The oscillations were more prolonged at the higher concentration, the frequencies being 0.091±0.005/min at 0.1 mmol/l and 0.067±0.005/min at 1.0 mmol/l. The $[Na^+]_i$ rhythmicity remained unaffected in the presence of 3 µmol/l tetrodotoxin but disappeared after addition of 10 µmol/l nifedipine or 400 µmol/l diazoxide. Oscillations of $[Na^+]_i$ induced by 11 mmol/l glucose were modified by 0.1 mmol/l tolbutamide with increase of the amplitudes from 4.9±0.3 to 7.4±0.7 mmol/l (n=15; P<0.005) and reduction of the frequency from 0.36±0.02 to 0.24±0.02/min (n=15; P<0.005). Conclusions: Tolbutamide is not only a potent stimulator of Na^+ entry into pancreatic β-cells but also mimics glucose in inducing rhythmic variations of the cytoplasmic concentration of Na^+.

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THE CALCIUM-SENSING RECEPTOR IN HUMAN ISLETS: CALCIUM-INDUCED INHIBITION OF INSULIN SECRETION.

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Aims: We have previously demonstrated that human β-cells express a functional calcium-sensing receptor (CaR). The purpose of the present study was to determine the effects of elevating extracellular calcium $[Ca^{2+}]_e$ on the insulin secretory profile of human islets of Langerhans. **Methods and Results:** In perifusion experiments, increasing the concentration of glucose from 2mM (0-10min) to 20mM (10-30min) evoked a rapid increase in insulin release from human islets (2mM glucose, 1.7±0.9 pg/islet/min, t=10min; 20mM glucose, 15.5±3.6, t=16min; $p<0.05$, $n=3$). In similar perifusion experiments, increasing $[Ca^{2+}]_e$ from 0.5mM to 5mM at 2mM glucose caused a rapid but transient rise in insulin secretion above basal secretion (0.5mM Ca^{2+}, 100±13.7%, t=0-10min; 5mM Ca^{2+}, 187.5±25%, t=14) followed by a significant inhibition of secretion below basal levels (5mM Ca^{2+}, 51.2±11.2%, t=20; $p<0.05$, $n=3$). A more pronounced inhibition was observed in the presence of 10mM Ca^{2+} (27.5±7.5% basal, $p<0.01$, $n=3$). A similar secretory profile was observed with increasing $[Ca^{2+}]_e$ at 20mM glucose, although the transient increase in secretion above basal induced by 5mM Ca^{2+} was not significant (0.5mM Ca^{2+}, 100±2.8%, t=0-10min; 5mM Ca^{2+}, 117±10%, t=12; $p>0.1$, $n=3$). The transient increase in secretion was followed by a concentration-dependent and prolonged inhibition of secretion (5mM Ca^{2+}, 65.7±10%; 10mM Ca^{2+}, 51.4±7.1%). At both 2 and 20mM glucose, the inhibition of secretion by Ca^{2+} was fully reversible. Incubation of human islets with increasing $[Ca^{2+}]_e$ at 2mM glucose caused small increases in the cyclic AMP content (0.5mM Ca^{2+}, 100±11.6%; 5mM Ca^{2+}, 159±18%; 10mM Ca^{2+}, 128±16%; $p<0.05$, $n=4$) while insulin secretion from the same islets was inhibited (0.5mM Ca^{2+}, 100±10%; 5mM Ca^{2+}, 51±2%; 10mM Ca^{2+}, 52±2%; $p<0.001$, $n=4$). **Conclusion:** This study has demonstrated that activation of the CaR inhibits basal and nutrient-stimulated insulin secretion, and that the inhibition is not mediated by Ca^{2+}-dependent decreases in cyclic AMP. This CaR-mediated inhibitory mechanism may be an important auto-regulatory mechanism in the control of insulin secretion.

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THE CALCIUM-SENSING RECEPTOR IN HUMAN ISLETS: CALCIUM-INDUCED INCREASES IN CYTOSOLIC CALCIUM.

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Aims: The purpose of the present study was to determine if human β-cells express a functional calcium-sensing receptor (CaR). **Methods and results**: Reverse transcription PCR using primers for the N-terminal sequence of the CaR expressed in human parathyroid-secreting cells identified a product of the expected size (374bp) in human pancreatic mRNA. Immunocytochemistry, using a monoclonal antibody against the extracellular C-terminal region of the CaR (NPS Pharmaceuticals, Salt Lake City), was used to immunostain 5μm sections of human pancreas. Double immunostaining with anti-somatostatin, glucagon, or insulin confirmed extensive immunoreactivity on α and β-cells, but not on δ-cells. Cells isolated from human islets (supplied by University of Leicester Human Islet Facility), which exhibited tolbutamide-evoked (100μM) increases in $[Ca^{2+}]_i$ at sub-stimulatory concentrations of glucose (2mM), also exhibited increases in $[Ca^{2+}]_i$ in response to elevated extracellular calcium ($[Ca^{2+}]_e$, 0.5 to 5mM; 26/32 cells in 9 experiments from 4 donors). The mean basal-to-peak Ca^{2+}-induced rise in $[Ca^{2+}]_i$ was 59% of the tolbutamide response. In 39% of those cells examined (N=10/26) the Ca^{2+}-evoked rise in $[Ca^{2+}]_i$ had returned to basal levels within 3 minutes of the original application, even in the continued presence of elevated concentrations (5mM) of $[Ca^{2+}]_e$. Ca^{2+}-induced increases in $[Ca^{2+}]_i$ were observed in some tolbutamide-insensitive cells (2/12 cells in 5 experiments from 4 donors). **Conclusion**: In human, islets of Langerhans, the CaR is expressed on insulin- and glucagon-containing cells, but not somatostatin-immunoreactive cells. Elevation of $[Ca^{2+}]_e$ evoked variable increases in $[Ca^{2+}]_i$. The CaR may link changes in $[Ca^{2+}]_e$ to insulin secretion via mechanisms independent of a sustained change in $[Ca^{2+}]_i$.

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PRESENILIN 2 IS LOCALISED IN ISLET β-CELLS BUT MET239VAL PS2 VARIANT IS NOT ASSOCIATED WITH DIABETES IN MAN

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Mutations of presenilin 1 and 2 (PS1,2) are causative factors for early-onset familial Alzheimer's disease and increased cerebral amyloid deposition. PS are expressed largely in the brain but PS2 is found also in muscle and pancreas. The function of PS are unknown but putative roles include protein trafficking, calcium homeostasis and apoptosis. **Aims:** To identify and localise PS2 in pancreatic tissues and to determine if PS2 is a candidate gene for diabetes by determination of the diabetic status of subjects with PS2 M239V mutation. **Methods:** Human and rodent pancreatic tissues were examined for PS2 by immunocytochemistry (ICC) and by Western blotting. Subjects of FLO10 pedigree (mean age 35y) with (n=5) and without M239V mutation (n=4) were tested with an OGTT. **Results:** Western blot analysis of extracts of rodent pancreas, mouse and human isolated islets, β-TC cells and human insulinoma demonstrated full length protein (50kDa) and fragments of 30 and 20kDa. PS2 was identified by ICC in islets, insulinoma and some pancreatic duct cells and in human foetal pancreas (12 weeks gestation). PS2 was present in amyloid-containing islets of Type 2 diabetic subjects but not in amyloid deposits. PS2 was identified in human islet β-cell granules and lysosomes. No evidence of glucose intolerance or increased secretion of proinsulin was found in subjects with M239V mutation. **Conclusions:** PS2 is a β-cell protein and could have a role in calcium homeostasis, trafficking of granule proteins including pro-islet amyloid polypeptide and pro-insulin.

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CATIONIC AND SECRETORY RESPONSE TO D-GLUCOSE IN DEPOLARIZED AND Ca^{2+}-DEPRIVED ISLETS EXPOSED TO DIAZOXIDE

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Aim. D-glucose stimulates insulin release from islets exposed to both diazoxide, to activate ATP-responsive K^+ channels, and a high concentration of K^+, to nevertheless cause depolarization of the B-cell plasma membrane. Under these conditions, the insulinotropic action of D-glucose is claimed to occur despite unaltered cytosolic Ca^{2+} concentration, but no information is so far available on the changes in Ca^{2+} fluxes possibly caused by the hexose. In the present experiments, we investigated the effect of D-glucose upon ^{45}Ca efflux from islets exposed to both diazoxide and high K^+ concentrations. **Methods.** Rat pancreatic islets prelabelled with ^{45}Ca were perifused, except if otherwise mentioned, in the presence of 0.25 mmol/l diazoxide and absence of Ca^{2+} (no $CaCl_2$, 0.5 mmol/l EGTA). **Results.** In the presence of diazoxide and at normal extracellular Ca^{2+} concentration, D-glucose (16.7 mmol/l) inhibited insulin release at 5 mmol/l K^+, but stimulated insulin release at 90 mmol/l K^+. In both cases, the hexose inhibited ^{45}Ca outflow. In the presence of diazoxide, but absence of Ca^{2+}, D-glucose (8.3 to 25.0 mmol/l) first caused a rapid decrease in insulin output followed by a progressive increase in secretory rate. This phenomenon was observed both at 5 mmol/l or higher concentrations (30, 60 and 90 mmol/l) of extracellular K^+. It coincided with a monophasic decrease in ^{45}Ca efflux and either a transient (at 5 mmol/l K^+) or sustained (at 90 mmol/l K^+) decrease in overall cytosolic Ca^{2+} concentration. **Conclusion.** In the absence of extracellular Ca^{2+}, D-glucose causes a secondary rise in insulin output from islets exposed to diazoxide. This effect, which is not linked to depolarization of the plasma membrane or Ca^{2+} entry, could be due to inhibition of Na^+-Ca^{2+} countertransport with resulting localized Ca^{2+} accumulation in the cell web of insulin-producing cells.

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Regulation of Islet Hormone Secretion

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Identification and Characterization of Single Glucagon-Secreting A-Cells in a Standard Mouse Islet Preparation

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Dispersed mouse pancreatic islet preparations are normally used for electrophysiological studies of B-cells, based on the high prevalence of this cell type in islets (>80%). A greater than average scatter in the results obtained from certain such preparations prompted us to routinely monitor the fractions of A- and B-cells by immunofluorescence microscopy. We obtained ratios of A:B in the range of about 7:1 to 1:50. The frequency of glucagon-positive A-cells in the preparation correlated with on average smaller cells (diameter and whole cell capacitance; ranging between 3.7+/-0.8 pF for high and 6.8+/-0.8 pF for low fraction of A-cells) and the occurrence of a TTX sensitive Na^+ current. A-cells could thus be identified during an experiment based on their electrophysiological properties and (to a limited extent) their appearance in phase contrast microscopy. A-cells displayed glucose-inhibitable electrical activity consisting of rapid action potentials that originated from a plateau of around –40mV and peaked at 0mV. The cells were equipped with a fast Na^+-carried transient, Ca^{2+}-currents that could be partially blocked by nifedipine (46+/-3%) and ω-conotoxin GVIA (23+/-4%), and an inactivating K^+-conductance ("A current"). Capacitance measurements revealed that A-cells were capable of Ca^{2+}-induced exocytosis. The exocytotic response to whole cell infusion of 2μM free Ca^{2+} was larger in A- than in B-cells from the same preparation (26+/-6fF/s vs. 4+/-1fF/s). Train-depolarizations separated a rapid initial component ("1st phase") and a slow component that activated with a delay of several seconds ("2nd phase"). Our results demonstrate that A-cells can be studied in a standard mouse islet cell preparation without the need for elaborate and potentially harmful cell sorting.

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PHOGRIN, AN INSULIN GRANULE MEMBRANE PTP HOMOLOGUE, IS PHOSPHORYLATED DURING STIMULATED INSULIN RELEASE.

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Reversible protein phosphorylation has frequently been implicated in the regulation of exocytosis and associated membrane trafficking events in the pancreatic β-cell. While the protein kinases involved have been studied extensively, little is known about their cellular substrates. Phogrin (IA-2 beta), a major target of autoimmunity in type I diabetes, is a 60/64 kDa integral membrane protein tyrosine phosphatase (PTP) homologue. It is localized to dense-core secretory granules, suggesting a possible physiological role in the regulation of granule biogenesis, exocytosis or granule movement during stimulated secretion. Here we show that phogrin is reversibly phosphorylated in intact pancreatic β-cells in response to a variety of secretory stimuli. In MIN6 cells, a mouse insulinoma line exhibiting the glucose sensitivity of islet β-cells, glucose dose-response and time course of phogrin phosphorylation paralleled the secretion of insulin. Both secretion and phosphorylation stimulated by either glucose or depolarizing concentrations of K^+ required the presence of extracellular Ca^{2+} ions. The calmodulin antagonist W-7 and the Ca^{2+}/calmodulin-dependent kinase II (CaM kinase II) inhibitor KN-93 dose-dependently inhibited both phosphorylation and secretion, with the "inactive" analogue KN-92 effective only at significantly higher concentrations. Phosphorylation of phogrin was also stimulated by exposure of cells to forskolin, which elevates intracellular concentrations of cyclic AMP (cAMP) and activates protein kinase A (PKA), a potent modulator of insulin secretion. The close correlation of phogrin phosphorylation with insulin release in response to different types of secretagogues is consistent with an involvement of this protein in the regulation of the secretory response at a site proximal to the exocytotic event.

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VISUALIZING INSULIN SECRETION WITH PH SENSITIVE GREEN FLUORESCENT PROTEINS

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Aims: Monitoring insulin secretion via visual methods has several advantages in the study of beta-cell function. First, the high temporal and spatial resolution of imaging techniques allows real time analysis of the trafficking of single secretory granules. Second, detection of insulin secretion from single cells allows an efficient analysis of the impact of microinjected oligonucleotides, antibodies, or impermeant inhibitors on insulin secretion. Visual techniques would be a significant improvement to currently used single cell assays including amperometry and plaque assays. Third, these visual techniques could be applied to permeablized beta-cell preparations and possibly isolated secretory granules. Fourth, in addition to quantitatively measuring insulin exocytosis, the design of appropriate probes facilitates measurements of secretory granule acidification, neutralization and recycling. **Materials and Methods:** pH sensitive green fluorescent proteins (pHluorins) have been recently engineered to visualize secretion in hippocampal neurons and the mast cell line RBL-2H3 (Miesenbock et. al., 1998, Nature 394:192). These pHluorins are targeted to the secretory granules by theVAMP-2 N-terminus and have different fluorescent properties at different pHs. The pH sensitivity of these proteins discriminates the unfused acidic secretory granule from the fused neutralized granule, therefore allowing dynamic measurements of exocytosis. We have applied this experimental design to assess insulin exocytosis in pancreatic beta-cells. **Results:** We have transiently and stably expressed these pHluorins in both MIN-6 and INS-1 beta-cell lines. We have demonstrated that the pHluorin protein co-localizes with insulin containing secretory granules. Neutralization of the pH in all vesicles with 50 mM ammonium chloride efficiently altered the fluorescent properties of the expressed pHluorins. The insulin secretagogues glucose and KCl each altered the fluorescent properties of these pH sensors in a dose-dependent manner. **Conclusion:** These results illustrate the potential use of these reagents for the study of pancreatic beta-cell exocytosis.

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CYTOSOLIC PHOSPHOLIPASE A_2 TRANSLOCATION AND ARACHIDONIC ACID-STIMULATED INSULIN SECRETION IN RODENT AND HUMAN β-CELLS.

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Arachidonic acid (AA) generation in β-cells following activation of the Ca^{2+}-dependent type IV cytosolic phospholipase A_2 ($cPLA_2$) may be a key factor in the coupling of nutrient-induced elevations in cytosolic Ca^{2+} to increased exocytosis of insulin. **Aims:** To determine the effects of increases in intracellular Ca^{2+} ($[Ca^{2+}]_i$) on β-cell $cPLA_2$ distribution and the effects of exogenous AA on insulin secretion from rat and human islets. **Methods:** The distribution of $cPLA_2$ in intact β-cells was assessed by immunofluorescence microscopy and in parallel experiments changes in $[Ca^{2+}]_i$ were measured by microfluorimetry using Fura 2-loaded cells. Insulin secretion was measured from islets in perifusion by radioimmunoassay. **Results:** Western blots of cytosolic and membrane fractions prepared from permeabilised rat islets maintained at a substimulatory Ca^{2+} concentration (50nM) detected a major $cPLA_2$-immunoreactive protein of approximately 95kDa which was almost entirely located in cytosolic fractions. Raising $[Ca^{2+}]_i$ to 10μM caused a rapid stimulation of insulin secretion accompanied by a movement of immunoreactive $cPLA_2$ from the cytosolic fractions. Confocal immunofluorescence microscopy indicated that under basal conditions $cPLA_2$ immunoreactivity was evenly distributed throughout the β-cell. Incubation for 1-30 minutes in the presence of tolbutamide (100μM) or KCl (20mM) caused rapid increases in $[Ca^{2+}]_i$ which were accompanied by a redistribution of $cPLA_2$-immunoreactivity. AA (50μM) caused a large increase in basal (2mM glucose) insulin secretion from both rat and human islets in perifusion, with a maximal 10-fold stimulation obtained after 10-12 minutes exposure to AA ($P<0.01$, n=3). **Conclusion:** These results indicate that elevations in $[Ca^{2+}]_i$ promote redistribution of $cPLA_2$ in β-cells and that the product of $cPLA_2$ activation stimulates insulin secretion.

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POSSIBLE INVOLVEMENT IN INSULIN SECRETION OF SYNAPSIN I AND Ca^{2+}/CALMODULIN KINASE II IN INSULINOMA CELL.

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Aims: Synapsin I is a synaptic vesicle-associated protein. Phosphorylation of synapsin I by Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II) correlates with neurotransmitter release. We examined the expression and functional role of synapsin I and isoforms CaM kinase II in insulinoma cells. **Materials and Methods**: We screened the MIN6 cDNA library with a probe of rat brain synapsin I cDNA. Isoforms of CaM kinase II expressed in MIN6 were examined by Northern blot analysis. Intracellular localization of synapsin I and CaM kinase II were examined by sucrose density gradient subfractionation and Western blot analysis. Secretagogues-induced phosphorylation of synapsin I, activation of CaM kinase II and insulin secretion were examined. **Results**: The largest open reading frame of 3,695 base pair encoded 670 amino acids with 98 % similarity to rat synapsin I. The expression of synapsin I was also confirmed in normal rat islets with reverse transcriptase-polymerase chain reaction analysis. MIN6 expressed β, γ and δ isoforms of CaM kinase II ($\delta > \beta > \gamma$). Western blot analysis after subcellular fractionation of MIN6 cells demonstrated that synapsin I and δ isoform of CaM kinase II were colocalized with insulin secretory granules. By stimulation with 25 mM glucose or 0.37 mM tolbutamide, phosphorylation of synapsin I increased by 25 or 68 %, respectively, in ^{32}P-labeled MIN6 cells. Under these conditions, the activity of CaM kinase II was elevated from 4.4 % (control) to 5.6 or 8.0 %, respectively. Insulin secretion from MIN6 cells correlated with the activity of CaM kinase II and increased from 13.2 μU/1x10^5 cells (control) to 17.3 or 20.2 μU/1x10^5 cells, respectively. **Conclusion**: Our results suggested that phosphorylation of synapsin I by δ isoform of CaM kinase II involved in the insulin secretion from islet beta cells.

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INSULIN SECRETION OF INS- 1 CELLS MODULATED BY CA2+/ CALMODULIN- DEPENDENT PROTEIN KINASE II

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Aims: Ca2+/calmodulin- dependent protein kinase II (CaM Kin II) is activated by glucose stimulation. Subtype δ_2 could be shown associated to insulin secretion vesicles. The role of CaM Kin II δ_2 in insulin secretion should be studied in INS- 1 cell- lines overexpressing CaM Kin II δ_2.

Materials: By the use of a retroviral system two INS- 1 cell- lines were constructed either with a stable or an inducible overexpression of CaM Kin II δ_2. For induction CaM Kin II δ_2 was constructed under control of the estrogen response element. INS- 1 cells or cell lines which contain the lacZ-Gen instead of CaM Kin II δ_2 were used as control. The level of expression was checked by immune blot using a polyclonal immune serum generated against the association domain of CaM Kin II δ_2. After stimulation with glucose 11 mM, glibenclamide 0,2μM or ionomycin 3μM insulin content in the supernatant was measured by standard RIA.

Results: Stably overexpression of CaM Kin II δ_2 does not significantly alter glucose or glibenclamide induced insulin secretion. In contrast, short- term overexpression of CaM Kin II δ_2 induced by the activation of the estrogen response element augmented Glibenclamide stimulated insulin secretion by 75 % and the response of insulin secretion to the Ca2+- ionophore ionomycin by 69%.

Conclusion: Glucose stimulated insulin secretion in INS- 1 cells is not influenced by overexpression of CaM Kin II δ_2. Glibenclamide stimulated insulin secretion in Ins- 1 cells is augmented by short- term overexpression of CaM Kin II δ_2 but not by a stably overexpression. The underlying mechanisms are not yet clear.

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ROLE OF NIFLUMIC ACID ON INSULIN SECRETION INDUCED BY EXCITATORY AND INHIBITORY AGONISTS IN MOUSE PANCREATIC ISLETS.

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Calcium-dependent K^+ (K_{Ca}) channels have been studied widely in various tissues, including endocrine cells. Recent studies using insulin secreting murine βTC-3 cells have suggested the presence of a Ca^{+2}-activated K^+ current. The channel was sensitive to block by clotrimazole and could be reversibly potentiated by niflumic acid. Our aim was to investigate a possible participation of that Ca^{+2}-activated K^+ channel in the regulation of insulin secretion by glucose in the presence of carbachol, UK 14.304, galanin and adenosine in mouse pancreatic islets. Insulin secretion was measured under static incubation. 20 μmol/l niflumic acid significantly inhibit insulin release induced by 15 mmol/l glucose (18.32±1.09 vs 28.15±1.84 μUI/islet/h., $p<0.001$). This inhibitory response was blocked by 1 μmol/l clotrimazole, whereas this antagonist did not modify insulin secretion induced by high glucose. When β-cells were stimulated by 50 μmol/l carbachol in the presence of 15 mmol/l glucose, insulin secretion was increased (36.92±1.47 μUI/islet/h., $p<0.001$ vs glucose). In this conditions niflumic acid attenuate insulin release evoked by carbachol (32.81±0.79 μUI/islet/h $p<0.05$ vs carbachol). However, the inhibitory responses mediated by 1μmol/l of the alpha-2 agonist UK 14.304 (percentage of inhibition vs glucose 52±3.11% $p<0.001$), 50 nmol/l galanin (58±3.02% $p<0.001$) and 500 μmol/l adenosine (42±2.3) were not affected by clotrimazole. It is conclude that this K_{Ca} channel sensitive to niflumic acid seems to negatively modulate insulin secretion evoked by the excitatory agonist but it is not involved in the inhibitory response under study.

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Insulin Secretion in Vitro

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SECRETORY HETEROGENEITY BETWEEN ISOLATED MOUSE ISLETS

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Secretory heterogeneity exists between sub-populations of isolated β-cells. It is not known, however, if such secretory variability also exists between intact islets. **Aim**: In order to determine if inter-islet secretory heterogeneity exists we have measured insulin release in response to glucose and GLP-1 from 132 islets. **Materials and methods**: Islets were isolated from NMRI mice, cultured overnight at 11 mM glucose and subsequently placed in microtiter wells, one islet per well. Insulin release during 30 min was measured in the presence of 3, 11 mM glucose and 11 mM glucose plus 10 μM GLP-1. **Results**: In the presence of 3 mM glucose average insulin release was 42 ± 9 pM. When the glucose concentration was augmented to 11 mM, insulin release averaged 289 ± 50 pM. Average insulin release was further increased to 655 ± 60 pM after addition of GLP-1. When insulin release was analyzed for each islet (n=132), variability between the islets was observed. Based on the secretory response to the increase of the glucose concentration from 3 to 11 mM, the islets were divided into three groups. "Low responders" increased insulin release about 50 %, "middle responders" approximately 5-fold and "high responders" more than 20-fold. The subsequent increase in insulin release in response to GLP-1 was 4-fold in "low responders", 3-fold in "middle responders" and 2-fold in "high responders". **Conclusions**: Islets show secretory heterogeneity in response to both glucose and GLP-1 stimulation. This variation may reflect heterogeneity in metabolism between islets and/or variations in the islet cell population of non-beta cells. Islets with a "low" secretory response to glucose augment secretion more vigorously upon further stimulation with GLP-1 as compared with "middle" or "high" responding islets. If such secretory heterogeneity also exists in vivo, it is possible that secretory less active islets, "low responders", are less vulnerable to β-cell toxins like streptozotocin and to autoimmune attack. Such islet heterogeneity could explain the selective destruction of islets during development of type 1 diabetes.

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PULSATILE INSULIN RELEASE FROM METABOLICALLY IMPAIRED MOUSE ISLETS

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Pulsatile insulin release has been connected with regular variations in metabolism. A decrease in the metabolic capacity of the β-cell has been implicated in the deranged secretory pattern in type 2 diabetes. **Aim**: To investigate how decreased metabolism by lowering the temperature affects the kinetics of insulin release. **Materials and methods**: Islets were isolated from the ob/ob mouse. Insulin release was measured from individual islets, perifused at 37° C in the presence of 3 and 11 mM. The temperature of the perifusion medium was gradually decreased to 20° C when 1 mM tolbutamide and, 20 min later, 1 mM dbcAMP were added. **Results**: Insulin release from individual islets at 37° C rose from 5.2 ± 0.5 to 144 ± 20 pmol*g^{-1}*s^{-1} when the glucose concentration of the perifusion medium was increased from 3 to 11 mM. The increase in secretion was achieved by augmentation of the amplitudes of the insulin oscillations without affecting their frequency (0.32 ± 0.08 osc/min). When the temperature of the perifusion medium was decreased, insulin release declined by reduction of the amplitudes of the pulses without affecting their frequency. When the temperature of the perifusion medium was 20° C the islets secreted approximately 1 pmol*g^{-1}*s^{-1}, which was the detection limit of the assay. At this point 1 mM tolbutamide was added to the perifusion medium which did not affect the secretory rate of the islets. Subsequently, 1 mM dibutyryl cyclic AMP was also added to the perifusion medium, which in combination with the sulfonylurea increased the pulsatile (0.35 ± 0.10 osc/min) release of insulin to 102 ± 18 pmol*g^{-1}*s^{-1}. **Conclusions**: The amplitude but not the frequency of the insulin oscillations from the isolated islet was affected when metabolism was impaired by lowering the temperature. Whereas addition of sulfonylurea did not affect insulin release under these conditions, the combined addition of sulfonylurea and cAMP almost restored pulsatile insulin release to levels observed at normal temperature.

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ASSOCIATION, BUT NOT LINKAGE OF IMPAIRED INSULIN- AND $[Ca^{2+}]_i$ RESPONSES TO GLUCOSE IN HUMAN PANCREATIC ISLETS

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AIMS: To compare the influence of long term elevated glucose and excessive stimulation in human islets on insulin and ($[Ca^{2+}]_i$) responses.

MATERIAL and METHODS: Human islets from seven donors were obtained from Beta Cell Transplant Unit, Brussels. Islets were cultured for 48 h at 5.5 or 27 mM glucose. Post-culture insulin and $[Ca^{2+}]_i$) responses were measured, the latter using the Fura-2 technique.

RESULTS: In 27 mM glucose-cultured islets the post-culture insulin response to glucose was totally obliterated. Basal $[Ca^{2+}]_i$ was elevated compared to 5.5 mM glucose-cultured islets (154.3±12.3 *vs* 95.9±7.3 nM, p<0.01). In islets cultured at 27 mM glucose the normal $[Ca^{2+}]_i$ response (peak from 95 to 165 nM) was totally abolished. In fact, in 3 of 11 islets a small decrease in $[Ca^{2+}]_i$ was observed after stimulation with high glucose. Slow oscillations (0.2-0.5 min^{-1}) analysed by Fast Fourier Transform were significantly reduced by culture at 27 compared to 5.5 mM glucose, p<0.05).

Diazoxide was added to culture media to investigate the importance of overstimulation. Diazoxide during culture decreased insulin accumulation in culture media by 63%. Previous diazoxide normalised post-culture insulin responses to glucose. Previous diazoxide also lowered basal $[Ca^{2+}]_i$ (123.6±8.6 *vs* 154.3±12.3 nM for islets cultured with and without diazoxide, p<0.03). In contrast, previous diazoxide did not to any extent restore post-culture stimulation by glucose of $[Ca^{2+}]_i$.

CONCLUSIONS: Long term exposure of human islets to elevated glucose in vitro abolishes both an insulin and a $[Ca^{2+}]_i$ response to glucose. Abolition of a $[Ca^{2+}]_i$ response is not corrected by previous diazoxide, which however restitutes insulin release. These results question the role of an abnormal $[Ca^{2+}]_i$ response for deficient signal transmission in human beta-cells.

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AUGMENTATION OF BASAL AND STIMULATED INSULIN SECRETION FROM RAT ISLETS BY THE GLUCOSE-INDUCED PRIMING EFFECT

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A previous short exposure to an elevated concentration of glucose augments the insulin secretory response of islets in response to a second stimulation with glucose or other insulinotropic agent. This enhancing effect, the priming effect of glucose was investigated. Prior exposure to glucose (above 8.3 mmol/l) produces enhancement not only of stimulated insulin secretion but also basal, both of which are induced dependent on glucose metabolism, because inclusion of 20 mmol/l mannoheputulose with 16.7 mmol/l glucose during the priming period abolished the enhancement. We also examined a late stage in the exocytotic process using electrically permeabilized islets, in which the intracellular Ca^{2+} and ATP concentration can be manipulated. Nonprimed or 16.7 mmol/l glucose-primed intact islets were electrically permeabilized and insulin secretion at various concentrations of Ca^{2+} and ATP was then examined. ATP dose-dependently augmented insulin secretion at clamped 1000 nmol/l Ca^{2+} from both nonprimed and primed islets which indicates ATP level may affect Ca^{2+} efficacy of exocytotic process in these islets. But, interestingly, the enhancement of insulin secretion from already primed islets was observed at any clamped concentrations of Ca^{2+} and ATP, especially in the presence of a low concentration (30 nmol/l) of Ca^{2+} and in the absence of ATP. These results indicate that the priming effect was derived, at least in part, from direct enhancement of Ca^{2+} efficacy in the exocytotic process of insulin granules, in which augmentation of Ca^{2+}- and the ATP-independent component of insulin secretion may play an important role. In addition, this component might explain the enhancement of basal insulin secretion from intact islets by glucose-induced priming.

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DO DIADENOSINE POLYPHOSPHATES PLAY AN OBLIGATORY ROLE IN GLUCOSE-STIMULATED INSULIN RELEASE ?

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Aim: Intracellular diadenosine polyphosphates (Ap_nA) were recently proposed as novel second messengers in nutrient-induced insulin release. Their accumulation in D-glucose-stimulated islets was reported to be prevented by NaF (50 µmol/l), an inhibitor of inorganic pyrophosphatase. The effect of NaF upon the metabolic, biosynthetic, cationic and secretory responses to D-glucose was now investigated in isolated pancreatic islets. **Methods:** The outflow of ^{86}Rb and ^{45}Ca from prelabelled perifused islets, the net uptake of ^{45}Ca, the oxidation of D-[U-^{14}C]glucose and utilization of D-[5-^{3}H]glucose, the incorporation of L-[4-^{3}H]phenylalanine into TCA-precipitable material and the release of insulin were measured in islets isolated from fed Wistar rats. **Results:** At concentrations up to 1.0 mmol/l, NaF failed to affect adversely the metabolism of D-glucose (16.7 mmol/l) and its effect upon ^{86}Rb and ^{45}Ca outflow, *de novo* biosynthesis of islet peptides and insulin release and only caused a modest inhibition of glucose-stimulated ^{45}Ca net uptake. At a 5.0 mmol/l concentration, however, NaF severely impaired the metabolism of D-glucose, as well as the biosynthetic and secretory responses to the hexose. Nevertheless, the glucose-induced inhibition of ^{86}Rb outflow was only partially decreased at the high concentration of NaF (5.0 mmol/l). **Conclusions:** Pending confirmation of the effect of NaF (50 µmol/l) on glucose-stimulated Ap_nA net production, the present findings do not suggest an obligatory role of these diadenosine polyphosphates in the stimulus-secretion coupling for glucose-induced insulin release.

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PHOSPHOCREATINE AND β CELL FUNCTION

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Changes in β cell energy state (eg the ATP/ADP ratio) are thought to be involved in the regulation of insulin secretion by glucose. Normal mouse islets were used to investigate the possible intervention of phosphocreatine (PCr) in this regulation. Immunocytochemistry showed that the brain isoform (CK-BB) of creatine kinase is abundant in β cells and apparently absent from non-β islet cells and exocrine cells. The muscle isoform (CK-MM) was weakly detectable in all cell types. Overnight culture of the islets with a physiological or pharmacological concentration of creatine (0.1 and 2 mmol/l) did not influence basal (3 mmol/l glucose) ATP content or ATP/ADP ratio, but increased the PCr content from 6 to 11 and 40 % of ATP levels, respectively. When creatine-treated islets were stimulated by 15 mmol/l glucose, their PCr content increased more than 2-fold and their ATP/ADP ratio doubled (as in controls). Raising islet $[Ca^{2+}]_i$ by tolbutamide or high K^+ similarly lowered the ATP/ADP ratio in control islets and in islets with a high PCr content. The latter also decreased in response to Ca^{2+}. Glucose-induced $[Ca^{2+}]_i$ changes had similar characteristics (phases, oscillations) and amplitude in control and creatine-treated islets. Insulin secretion was also unaffected. The results thus show that PCr levels are low in islet cells, even after culture with a physiological creatine concentration. Their pharmacological increase does not efficiently buffer adenine nucleotides and does not alter events (secretion, $[Ca^{2+}]_i$ oscillations) linked to adenine nucleotides changes. The parallelism of PCr and ATP/ADP changes induced by glucose and Ca^{2+} indicates that the observed adenine nucleotide changes likely occur in the cytoplasm of β cells.

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β-CELL OUABAIN-SENSITIVE $^{86}Rb^+$ INFLUX (Na^+/K^+ PUMP): EFFCTS OF D-GLUCOSE, GLIBENCLAMIDE OR DIAZOXIDE

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Aims: To study whether the Na^+/K^+ pump (ouabain-sensitive $^{86}Rb^+$ influx) is affected by D-glucose, glibenclamide or diazoxide. **Materials and Methods:** To estimate the activity of the Na^+/K^+ pump in intact collagenase-isolated ß-cell-rich islets from ob/ob mice, $^{86}Rb^+$ influx was measured in the absence or presence of 1 mM ouabain. **Results:** We have previously studied the activity of the Na^+/K^+ pump in intact ß-cells. Those experiments showed that D-glucose (20 mM) significantly stimulated the ouabain-sensitive portion of $^{86}Rb^+$ influx by >60%, whereas the ouabain-resistant portion (including e.g. K^+ channels) was inhibited. The present results showed that the stimulatory effect of glucose reached its approximate maximum at 5 mM glucose ($P<0.005$; n=14) and did not further increase up to 20 mM sugar. Pretreatment (60 min) of islets with 20 mM glucose dramatically reduced the glucose-induced stimulation of the Na^+/K^+ pump (34% glucose-induced increase ($P>0.05$; n=10) after 20 mM glucose pretreatment as compared with 74% glucose-induced increase ($P<0.001$; n=10) after pretreatment in the absence of glucose; ($P<0.01$; n=10) for the difference between 34% and 74%). Long-term pretreatment (180 min) of islets at 0 mM glucose, on the other hand, did not affect the level of glucose-induced stimulation of $^{86}Rb^+$ influx during the subsequent 5-min incubation. Since previous results have suggested that sulphonylureas inhibit the activity of the islet Na^+/K^+ ATPase, we studied the effect of glibenclamide (0.1 to 10 µM) on the $^{86}Rb^+$ influx. Glibenclamide stimulated the Na^+/K^+ pump in the same manner as glucose. The stimulatory effect was evident at 0.5 µM (27% increase; $P<0.05$; n=6). Diazoxide (0.4 mM) inhibited the Na^+/K^+ pump by 38% ($P<0.005$; n=15). The stimulatory effect of glibenclamide was totally abolished by diazoxide. **Conclusions:** The results show that both nutrient and non-nutrient insulin secretagogues, which act by depolarising the ß-cell membrane, also stimulate the ouabain-sensitive $^{86}Rb^+$ influx. This suggests that those secretagogues activate the Na^+/K^+ pump, probably as part of the membrane repolarisation process.

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CELL-CELL CONTACT AND INSULIN SECRETION IN MIN6 PSEUDOISLETS

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Aims: The effect of cell-cell contact on insulin secretion was studied using MIN6 pseudoislets, three-dimensional cell aggregates, which develop when MIN6 cells are cultured on gelatin. **Materials and Methods**: Insulin secretion from late passage MIN6 monolayer cells (M) and pseudoislets (PI) was measured by RIA after 1 hour incubation. **Results:** While only a small change in secretion was observed in M in response to increasing concentrations of glucose (2 mM glucose (basal): 100±13%; 7.5mM: 113±11, 10mM: 143±9; 15mM: 135±6; 20mM: 149±8, n=8; $P<05$ by ANOVA, mean±SEM) PI released insulin in a manner similar to primary islets (2mM glucose: 100±21; 7.5mM: 213±19; 10mM: 377±20; 15mM: 436±21; 20mM: 418±10, n=8, $P<0.0001$ by ANOVA). Marked differences in insulin release were also seen in response to 10 mM KIC (M: 114±48, n=19; PI: 470±107, n=11, $P<0.05$) and to a range of non-nutrients (20 mM KCl: M: 162±12% basal, n=6, PI: 382±84, n=4, $P<0.05$; 100µM tolbutamide: M: 138±10% basal, n=11; PI: 423±138, n=7; $P<0.05$; 10 µM FSK: 115±7% of secretion at 20 mM glucose, n=9; PI: 354±39, n=12, $P<0.001$; 500 nM PMA: M: 113±12, n=9; PI: 445±50, n=12, $P<0.001$). Incubation in the presence of heptanol (2.5 mM), a gap junctional blocker, reduced insulin release in PI (2 mM glucose: 57±7% of control, n=5; 20mM; 39±8, n=5, 100µM tolbutamide: 21±2, n=3) but not in M (2 mM glucose: 145±6 of control, 20 mM 139±6, 100 µM tolbutamide: 100±8, n=8). **Conclusion:** These results suggest that close cell-cell contact within the pseudoislet improves the secretory responsiveness of MIN6 cells and that this may be due to gap junctional communication. Pseudoislets may therefore serve as an improved research model for the study of β-cell function in general and cell-cell communication in particular.

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Prolongation of islet survival by pefloxacin during storage under hypothermic conditions.

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The aim of the present study was to exam the effect of pefloxacin on secretory function of isolated pancreatic islets stored for 24 hours under hypothermic conditions. **Materials and methods:** Rat pancreatic islets were isolated by collagenase digestion. Experiments were performed on four groups of islets. Freshly prepared islets served as controls (group I). Group II consisted of islets stored in Hanks solution (HBSS). Group III included islets isolated from rats pre-treated with PFX given i. v. at the dose of 6 mg/kg bw 2 hours prior isolation and stored in HBSS. In group IV islets isolated from non-pre-treated donors were stored in HBSS supplemented with pefloxacin at the concentration of 40 μg/ml. Islets from group II - IV were stored at 4°C for 24 hours. Insulin release from islets was examined during incubation. For the first 45 min incubation medium contained 3 mM glucose (basal release), then it was changed to a concentration of 20 mM glucose plus 1 μM forskolin (stimulated release) for another 45 min followed by a return to the low glucose concentration. **Results:** Stimulated insulin secretion during incubation was analysed using the stimulation index (SI) defined as the ratio of stimulated to basal insulin release. Freshly prepared islets increased the rate of insulin secretion during stimulation (2.5±0.4 vs 13.2±2.4 ng/45 min/islet; mean SI 5.3±0.5). After the 24-hour storage islets from group II did not increase the rate of insulin release under stimulatory conditions (2.6±0.8 vs 2.4±0.6 ng/45 min/islet). A significant response to 20 mM glucose plus forskolin challenge was found for group III and IV (1.8±0.3 vs 5.6±0.8 ng/45 min/islet; mean SI 3.6±0.9 and 1.1±0.2 vs 2.6±0.3 ng/45 min/islet; mean SI 3.3±0.6, respectively). **Conclusions:** The results of the present study suggest that pefloxacin limits pancreatic islet injury and prolongs islet survival during storage.

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EFFECTS OF GADOLINIUM ON ION CURRENTS AND INTRA-CELLULAR CALCIUM OF MOUSE PANCREATIC B-CELLS

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Aims: Transplantation of islets or insulin producing cells into the liver may induce the activation of Kupffer cells and thus a repulsion or destruction of the graft. Systemic application of gadolinium (Gd^{3+}) can prevent the activation of Kupffer cells resulting in a longer survival of islet grafts. Since Gd^{3+} is an unspecific inhibitor of non-selective cation currents we tested Gd^{3+} for an effect on ion currents of mouse pancreatic B-cells which are involved in stimulus-secretion coupling. **Materials and Methods:** Ion currents were measured with single B-cells cultured up to 4 d. The holding-potential was -70 mV; K^+_{ATP} currents were determined by 10 mV depolarizing steps, voltage-dependent currents during steps to 0 mV. $[Ca^{2+}]_i$ was measured by fura-2 fluorescence. **Results:** Gd^{3+} (100 μM) inhibited the K^+_{ATP} current from 206±22 pA under control conditions to 163±24 pA (n=8). In 6 out of these experiments a transient increase to 245±33 pA was observed first. Voltage-dependent K^+ currents were also diminished by Gd^{3+}. The current was 705±126 pA (control) and was reduced to 246±57 pA (n=7) by Gd^{3+} (100 μM). Gd^{3+} also decreased the voltage-dependent L-type Ca^{2+} current even at low concentrations. Under control conditions the Ca^{2+} current amounted to -84±14 pA (n=8). It was reduced to -32±6 pA (n=8) and to virtually zero (-1±1 pA, n=8) by 0.1 μM and 1 μM Gd^{3+}, respectively. Consequently, Gd^{3+} (100 μM) also reduced $[Ca^{2+}]_i$ measured at a stimulatory glucose concentration of 15 mM to basal levels (n=4). **Conclusions:** The blockade of the Ca^{2+} currents by Gd^{3+} and the resulting decrease in $[Ca^{2+}]_i$ undoubtedly lead to an inhibition in insulin secretion of pancreatic B-cells. Thus, the positive effect of Gd^{3+} on the survival rate of islet grafts may be counteracted by these mechanisms.

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The Role of Mitochondria in Stimulation of Insulin Secretion

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EFFECTS OF ALLOXAN ON MEMBRANE POTENTIAL, Ca^{2+}-ACTIVITY AND MITOCHONDRIAL MEMBRANE POTENTIAL OF PANCREATIC B-CELLS

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Aims: Alloxan which is used to induce diabetes chemically impairs B-cell function probably via production of reactive oxygen species (ROS), including H_2O_2. However, the mechanism is still a matter of debate. Thus, we investigated the effects of alloxan on the membrane potential, intracellular calcium activity ($[Ca^{2+}]_i$) and mitochondrial membrane potential ($\Delta\Psi$). **Materials and methods:** All experiments were performed with mouse pancreatic B-cells. The membrane potential was recorded with intracellular microelectrodes in intact islets. Measurements of $[Ca^{2+}]_i$ and $\Delta\Psi$ were made with large clusters of cells, loaded with the fluorescence dyes fura-2 and rhodamine 123, respectively. **Results:** 1 mM alloxan hardly affected the membrane potential (n=4), while 5 mM alloxan led to an irreversible hyperpolarization (n=5), which could only be counteracted by treatment with tolbutamide (n=6). However, the sensitivity of the cells to tolbutamide was reduced. To ensure that effects of 1 mM alloxan were not prevented by a glucose concentration of 15 mM, we added 1 mM alloxan in the presence of 3 mM glucose. Subsequent treatment with 15 mM glucose led to a normal depolarization with slightly reduced spike activity (n=6). Pretreatment with a high glucose concentration (45 mM) did not prevent the hyperpolarization of the membrane potential induced by 5 mM alloxan. $[Ca^{2+}]_i$ was first decreased by 5 mM alloxan, however the decrease was followed by a sustained rise (n=6). $\Delta\Psi$ was clearly depolarized by 5 mM alloxan (n=6). All these effects resemble those of H_2O_2 reported earlier. **Conclusions:** The depolarization of $\Delta\Psi$ indicates a decrease in ATP synthesis which probably leads to the observed hyperpolarization of the membrane potential. This hyperpolarization is supposed to lead to suppression of insulin secretion. The observed similarities between H_2O_2 and alloxan on membrane potential, $[Ca^{2+}]_i$ and $\Delta\Psi$ emphasize the view that alloxan indeed alters B-cell function by production of H_2O_2.

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OXIDATIVE STRESS ALTERS MITOCHONDRIAL METABOLISM, CELLULAR Ca^{2+} POOLS AND INSULIN SECRETION.

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Aims: The mitochondria exert a key role in the control of nutrient induced insulin exocytosis and are also the main source of oxidants through generation of hydrogen peroxide (H_2O_2), which may impair β-cell signal transduction. **Methods**: The β-cell line INS-1 was exposed to 200μmol/L H_2O_2 for 10min before measuring several parameters including $[Ca^{2+}]$ and [ATP] in cells expressing the Ca^{2+}-sensitive photoprotein aequorin (organelle targeted) or luciferase, respectively. **Results**: Insulin secretion stimulated by glucose (12.8 vs. 2.8mmol/L; 3-fold; $p<0.001$) was abolished after exposure to H_2O_2, which also resulted in elevated basal insulin release (2-fold; $p<0.02$). After the oxidative stress, glucose was no longer able to depolarize the cell membrane potential ($\Delta\Psi$), nor to increase cytosolic $[Ca^{2+}]$. Both $\Delta\Psi$ and $[Ca^{2+}]$ responses were still observed with KCl despite augmented cytosolic $[Ca^{2+}]$ appearing only approx. 10min after H_2O_2 treatment. The mitochondrial $\Delta\Psi$ of INS-1 cells was depolarized by the oxidative stress abolishing the hyperpolarizing action of glucose. These $\Delta\Psi$ changes correlated with altered mitochondrial morphology, which was not preserved by the overexpression of the antiapoptotic protein Bcl-2. Mitochondrial $[Ca^{2+}]$ was increased by H_2O_2 up to the μmolar range and no further augmentation was observed upon glucose addition, which normally raises this parameter. Cytosolic [ATP], was markedly and rapidly reduced by H_2O_2 treatment (-57%; $p<0.001$). These changes preceded the decrease in endoplasmic reticulum $[Ca^{2+}]$ (lag time 47±4sec) which accounts for the subsequent elevation in cytosolic $[Ca^{2+}]$. **Conclusions**: The present results point to the mitochondria as the primary target for oxidative stress damage with consequences on cellular Ca^{2+} pools and the normal coupling of glucose metabolism to insulin secretion.

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OSCILLATIONS IN INTRACELLULAR CALCIUM PROVOKE OSCILLATIONS IN THE MITOCHONDRIAL MEMBRANE POTENTIAL OF B-CELLS

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Aims: Oscillations in B-cell activity are a prerequisite for the normal pulsatile insulin secretion and are impaired in diabetes mellitus. These oscillations are thought to be owing to oscillations in metabolism, but the underlying mechanisms are unclear. This study wanted to further elucidate the role of mitochondria in the oscillations. **Methods:** Cell membrane potential (MP) and K^+_{ATP} currents were measured in the perforated-patch mode in mouse pancreatic B-cells. $[Ca^{2+}]_i$ was determined by the fura-2 technique and the mitochondrial membrane potential ($\Delta\Psi$) by rhodamine123 fluorescence. **Results:** Increasing the extracellular glucose concentration from 0.5 to 15 mM led to the well-known biphasic change and eventually to oscillations in $[Ca^{2+}]_i$ (n=16). The same manoeuvre first hyperpolarized $\Delta\Psi$ (n=26) reflecting the increase in ATP production which underlies the initial drop in $[Ca^{2+}]_i$. As soon as $[Ca^{2+}]_i$ increased this was followed by a depolarization of $\Delta\Psi$ (n=21) and thereafter by synchronous oscillations in $[Ca^{2+}]_i$ and $\Delta\Psi$ (n=16). These oscillations were stopped by cyclosporin A (CsA, 2 µM) an inhibitor of the permeability transition pore (PTP) (n=5). Consequently, CsA (2-5 µM) also inhibited glucose-induced electrical activity, however, without a hyperpolarization to the resting MP or an increase in K^+_{ATP} current (n=4) as it was observed with mitochondrial uncouplers, e.g. NaN_3 (n=3) or FCCP (n=4). The Ca^{2+}-induced depolarization of $\Delta\Psi$ was not owing to glucose metabolism, because it also occurred when the cells were treated with a high extracellular K^+ concentration (30 mM) at a substimulatory glucose concentration (5 mM) (n=4). **Conclusions:** The glucose-induced increase in $[Ca^{2+}]_i$ and Ca^{2+} uptake into the mitochondria may open the PTP thus depolarizing $\Delta\Psi$ and decreasing ATP production. This mechanism is proposed to serve as a feedback loop in stimulus-secretion coupling, explaining oscillations in B-cell activity.

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SWITCH TO ANAEROBIC GLUCOSE METABOLISM BY ACCUMULATED NADH IN THE ß-CELL MODEL OF MITOCHONDRIAL DIABETES.

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Aim To elucidate the mechanism underlying diabetes caused by mitochondrial gene mutation, we created its model by applying low dose (0.4 µg/ml) ethidium bromide (EB) to a mouse pancreatic ß cell line ßHC9 for 4-6 days. In this system, we reported that transcription of mitochondrial DNA, but not that of nuclear DNA, was time-dependently inhibited accompanied by suppression of glucose-stimulated insulin release. To fully understand the effect of the lowered transcription of mitochondrial DNA on glucose metabolism, we performed the following experiments. **Methods** Glucose oxidation and utilization were measured using [^{14}C(U)]- and [5-^{3}H]-glucose, respectively. Lactate production was measured by lactate dehydrogenase method. Autofluorescence of NADH of monolayer-cultured cells was determined using 2 photon-excited laser microscopy. Control experiments were done using cells cultured simultaneously without EB over the same duration. Statistical analyses were performed by Student's *t* test. **Results** [1] Glucose (22.2 mM) oxidation was severely decreased by EB treatment compared with the control cells (by 63% on day 4 and by 78% on day 6; both $p<0.01$). [2] By contrast, glucose (22.2 mM) utilization was only marginally affected (decreased by 8-16%). [3] Lactate production under 22.2 mM glucose was increased by 180% and 250% by EB on day 4 and 6, respectively (both $p<0.01$). [4] Cellular NADH concentrations at 2.8 mM glucose were increased by 35% and 43% by EB on day 4 and 6, respectively (both $p<0.01$). **Conclusions** These data suggest that low expression of mitochondrial electron transfer system including NADH dehydrogenase due to suppressed mitochondrial DNA transcription causes NADH accumulation in the ß cells, thereby halting TCA cycle and fascilitating anaerobic glucose metabolism. These biochemical features are well correlated to the clinical features of mitochondrial diabetes.

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METHYL-PYRUVATE INITIATES INSULIN RELEASE BY A K^+_{ATP}- CHANNEL INDEPENDENT MECHANISM

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Aims: Mitochondria play an important, albeit unclear role in stimulus-secretion coupling of pancreatic ß-cells. Thus pyruvate, which is a pure mitochondrial substrate, does not induce insulin secretion whereas methyl-pyruvate (MP) is insulinogenic. This study aims at further elucidating this difference in the mode of their action. **Methods:** Electrical activity and insulin secretion in response to pyruvate and its methyl ester were compared. **Results:** In rodent islets pyruvate (20 mM) failed to stimulate insulin release and no effect on membrane potential was observed. However, MP significantly stimulated insulin secretion in the absence of glucose from 1.97±0.28 to 8.13±1.06 ng ml^{-1} 60 min^{-1} (n=8-10, $p<0.05$) and in the presence of 3 mM glucose from 3.44±1.29 to 15.34±5.49 ng ml^{-1} 60 min^{-1} (n=3-5, $p<0.05$). Insulin secretion was parallelled by membrane depolarisation showing continuous spiking activity. The sustained depolarisation level induced by 20 mM MP remained unaffected by the addition of the Ca^{2+}-channel antagonist isradipine (1 µM), however, spiking activity was completely suppressed. Azide (3 mM) hyperpolarised the ß-cell both in the presence of glucose (16 mM) or MP (20 mM). Membrane depolarisation induced by MP (20 mM) was insensitive towards the addition of diazoxide (100 µM) whereas glucose induced membrane depolarisation was suppressed. **Conclusion:** It is suggested that MP-induced membrane depolarisation and insulin release are independent of K_{ATP}-channel activity.

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PIVITOL ROLE OF LACTATE DEHYDROGENASE ACTIVITY IN THE CONTROL OF MITOCHONDRIAL METABOLISM OF MIN6 β-CELLS.

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Aims: Low lactate dehydrogenase (LDH) activity is important for normal regulation by glucose of islet β-cell insulin release. We have determined whether overexpression of the LDH-A isoform affects insulin secretion in single islet MIN6 β-cells by interfering with mitochondrial oxidative metabolism.

Materials and Methods: MIN6 cells were co-microinjected with plasmids encoding LDH-A and enhanced green fluorescent protein (to identify injected cells), prior to measurements by confocal microscopy of NAD(P)H, using autofluorescence, or mitochondrial membrane potential ($\Delta\psi_m$), using the fluoresecent potential-sensitive dye, tetra-methyl rhodamine ethyl ester (TMREE). Alternatively, cells were microinjected with firefly luciferase cDNA with or without LDH cDNA, and cytosolic [ATP] monitored through single cell luminescence changes.

Results: In response to an increase in glucose concentration from 3 mM to 30 mM the following increases in the measured variables were observed :

	NAD(P)H *fluorescence (340 nm)*		$\Delta\psi_m$ *TMREE fluorescence*		cytosolic [ATP] *bioluminescence*	
Time	90s	360s	90s	360s	90s	360s
Control	18 ± 5%	34 ± 9%	11 ± 2%	41 ± 12%	4.4 ± 1.7%	7.6 ± 2.7%
LDH-A	14 ± 3%	16 ± 5%*	9 ± 1%	21 ± 9%*	4.3 ± 2.8%	0.5 ± 3.3%*

(n = 3 preparations, * $p<0.05$, 1-tailed t-test compared to control cells).

Conclusions: Overexpression of LDH-A in MIN6 cells diminished glucose-induced increases in mitochondrial intermediates and cytosolic ATP. Intriguingly, the observed differences caused by LDH-A expression were more pronounced with regard to the second (>200s) phase of metabolic stimulation than the initial 'burst'. These results may suggest that dysregulation of LDH genes could contribute to some forms of non-insulin dependent diabetes mellitus.

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DEXAMETHASONE-INDUCED CHANGES IN FAD-GLYCEROPHOSPHATE DEHYDROGENASE EXPRESSION IN HUMAN PANCREATIC ISLETS.

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Several works have drawn attention to an unfavourable effect of corticosteroid hormones on the secretory activity of islet B-cells.The mitochondrial FAD-glycerophosphate dehydrogenase plays a key role in the process of glucose-induced insulin release from pancreatic islet β-cells. **Aim:** To assess the effect of D-glucose (D-G) and dexamethasone (DX) upon mGDH mRNA and protein expression, mGDH activity, as well as, glucose-stimulated insulin release in human pancreatic islets. **Methods**: Human pancreatic islets were cultured for 63 hours at 2.8 or 16.7mM D-G and, as required, 10nM-10μM DX. mGDH mRNA and protein levels were analysed by Northern and Western blot techniques respectively. **Results**: Human pancreatic islets cultured for 63 h at 2.8mM D-G in the presence of 10nM-0.1μM and 1.0-10μM DX caused a concentration-related decrease in the mGDH mRNA expression, averaging 102.4±5.3% (n=4) and 75.9±5.4% (n=6; p<0.01) respectively of the paired control value found after culture in the absence of DX. At 16.7mM D-G concentration, the incorporation of 10nM-0.1μM and 1.0-10μM DX into the culture medium decreased such a percentage to respectively 88.0±3.5% (n=3) and 66.3±5.5% (n=6;p<0.005). DX (1.0 to 10μM) also decreased the mGDH protein content in islets cultured in the presence of 2.8 or 16.7mM D-G. The values recorded after culture in the presence of 1.0 and 10μM DX averaged respectively 72.7±2.4 (n=6) and 25.4±1.0 (n=6 in both cases) of the paired control measurement. Likewise, the catalytic activity of mGDH was decreased in a concentration-related manner by DX (10nM to 1μM) in islets cultured at 16.7mM D-G. After exposing the islets to 10nM,0.1μM and 1.0μM of DX, the enzymatic activity values averaged respectively 105.3±12.6, 75.1±7.8 and 53.9±5.6% (n=3 in all cases; p<0.05) of the paired control value. DX failed to affect significantly the activity of the enzyme in islets cultured at 2.8mM. The insulinotropic action of D-glucose was suppressed by DX. **Conclusion**: DX impairs the mGDH mRNA content, mGDH protein content and mGDH activity, as well as, glucose stimulated insulin release in human islets cultured for 63 h in the presence of DX. These effects are more pronounced in islets cultured at 16.7mM rather than 2.8mM D-G.

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METABOLIC AND FUNCTIONAL STUDIES ON ISLETS ISOLATED FROM A NEW RAT MODEL OF TYPE 2 DIABETES

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Aims: In order to further characterize the features of a new model of type 2 diabetes obtained in adult rats given streptozotocin (STZ) plus nicotinamide (NA), morphological, metabolic and functional studies were performed on islets isolated from diabetic rats. Materials and Methods: Four-six weeks after induction of diabetes (STZ 60 mg/kg i.v. given 15 min after i.p. injection of 280 mg/kg NA), morphometric analysis was carried out on the whole pancreas. Islets were isolated by the collagenase method and used to assess functional capabilities in static incubations, glucose metabolism (by measurement of radiolabeled glucose usage and oxidation) and mitochondrial glycerophosphate dehydrogenase (mGDH) protein levels (by immunoblotting) and activity (by a radioisotopic procedure). Results: With respect to control islets, in diabetic islets the following changes were observed: a 36% decrease in the number of β cells and no change in α cells; a decrease in both mGDH content (-77%) and activity (-60%); a slight reduction in glucose utilization and no change in global glucose oxidation. Insulin release stimulated by 16.7 mM glucose was decreased in diabetic islets (0.83 ± 0.04 vs. 1.28 ± 0.09 ng/islet/h in control islets, $p < 0.01$), as was that stimulated by 16.7 mM glucose plus 1 mM IBMX. However, the insulin responsiveness to other stimuli (8.3 mM glucose, 2-ketoisocaproate, D-glyceraldehyde) was unchanged. The inhibitor of malate-aspartate shuttle aminooxyacetate (5 mM) reduced D-glyceraldehyde-stimulated insulin release more in diabetic than in control islets. Conclusions: In STZ plus NA-diabetic rats, a decrease in β cell mass occurs, as well as a marked reduction in the amount and activity of islet mGDH, the key enzyme of the glycerol phosphate NADH shuttle. From our functional studies, it appears that these alterations do not affect islet glucose metabolism and insulin responsiveness as much as it could be expected; in particular, mGDH deficiency results in secretory abnormalities only in the case of blockade of the other NADH shuttle system operative in islets.

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NO and Insulin Secretion

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INCREASED NITRIC OXIDE LEVELS AND FIRST-PHASE INSULIN SECRETION ARE EARLY FEATURES OF DIET-INDUCED INSULIN RESISTANCE IN RATS.

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Aim: To investigate whether a high-lipid and/or high-sucrose diets are able to increase nitric oxide levels (NO_2^-/NO_3^-) and first-phase insulin secretion. **Materials and Methods**: Rats were fed with different diets: control diet (CT), high-lipid diet (LIP) and high-sucrose plus lipid diet (SU) for 2 and 8 weeks. At the end of these periods, each rat underwent an IVGTT (0.3 g/Kg BW) and blood samples were withdrawn at -5, 2, 4, 6 and 8 minutes. **Results**: After 2 weeks FFA, triglyceride and nitric oxide levels were higher in LIP and SU diets than CT diet without significant differences between the two diets (p<0.05). Fasting insulin levels were significantly increased in SU but not in LIP diet (p<0.05) while first-phase insulin secretion was similarly increased in both diets (p<0.05). After 8 weeks, a progressive increase of retroperitoneal fat (5.7±0.5, 18.2±1.6 and 22.8±1.4 g; p<0.05), insulin (45.4±15.7, 114.4±17 and 172.8±62,1 pmol/l; p<0.05), glucose (68.7±1.9, 81.8±4.5 and 109.3±29.0 mg/dl; p<0.05), FFA (0.7±0.03, 1.1±0.1 and 1.3±0.1 mmol/l; p<0.05), triglyceride (52.6±5.6, 75.0±10.0 and 116.2±21.8 mg/dl; p<0.05), nitric oxide levels (2.8±0.4 , 5.7±0.2 and 8.5±0.8 μmol/l; p<0.05) and first-phase insulin secretion (225.2±194.0, 371.1±136.6 and 1092.7±257.0 pmol/l x min; p<0.05) was found. Positive correlations were demonstrated between nitric oxide levels and fasting insulin (r=0.771, p<0.0004) and first-phase insulin secretion (r=0.49, p<0.03). At multiple regression analysis first-phase insulin secretion was independently related to retroperitoneal fat, fasting blood glucose, serum triglyceride and nitric oxide levels. **Conclusions**: SU diet and/or LIP diet induce a progressive state of insulin resistance and an early increment of nitric oxide levels. Furthermore, nitric oxide levels, among other indices of insulin resistance, independently correlates with the increased first-phase insulin secretions.

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CONSTITUTIVE NO SYNTHASE ACTIVITY CAN MODULATE INSULIN SECRETION IN THE INS-1 CELL LINE.

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Aims: Since the presence of a constitutively expressed nitric oxide synthase (cNOS) in pancreatic β cells is still a matter of controversy, the present work was aimed at investigating whether cNOS activity could exert a control in insulin secretion in the glucose-responsive cell line INS-1. Materials and Methods: Cultured INS-1 cells (7 x 10^5 cells per well) were incubated for 1 h in KRB/Hepes buffer, containing 0.2% BSA and 2.8 mM glucose, to explore the insulin secretory effects of L-Arginine (the cNOS physiological substrate), L-N-ω-Nitro-L-Arginine Methyl Ester (L-NAME, a competitive inhibitor of cNOS), as well as D-NAME and KCl to reveal possible non specific effects. All compounds were tested at increasing concentrations (0.67, 2, 5 and 10 mM), either alone or using different combinations. Results: All the four substances exerted stimulating effects at 10 mM, which suggests involvement of a depolarizing component at this concentration. However, they acted differently at lower concentrations. L-NAME, the most effective at 10 mM (insulin release was 19.5 ± 1.4 ng/well/h versus 8.1 ± 0.4 at 2.8 mM glucose alone, p<0.01), was also able to stimulate insulin secretion at 2 and 5 mM (+ 27 and + 52% over basal values, respectively, p<0.05), which could not be found for KCl and D-NAME, thereby suggesting an inhibitory tone of cNOS on basal insulin secretion. In further support of the modulatory action of cNOS activity in INS-1 cells, arginine, substrate of cNOS, was found to significantly decrease (-30% at 0.67 and 2 mM ; -40% at 5 and 10 mM, p<0.05) not only the marked stimulating effect of L-NAME (10 mM), but also that of 10 mM KCl (-15% at 0.67 mM, -28% at 2 mM, p<0.05). These functional data are strongly supported by immunocytochemical studies, performed by using a polyclonal antibody directed against rat neuronal cNOS, which showed the presence of this isoform of the enzyme in the INS-1 cells. Conclusions: Taken together, our results demonstrate that a neuronal cNOS isoform is expressed in the INS-1 cell line and may contribute to modulate insulin secretion.

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NITRIC OXIDE ACTS AS A DIFFUSIBLE SYNCHRONIZER OF PANCREATIC β-CELLS

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Aims. Although gap junctional coupling is important for co-ordinating the activity of the pancreatic β-cells, there is evidence for the involvement also of a diffusible factor. We have now investigated whether nitric oxide serves as a diffusible synchronizer of the β-cells. Materials and Methods. The studies were performed with isolated mouse β-cells cultured for 2-5 days before superfusion under the microscope. Digital imaging of the fura-2 fluorescence was used to simultaneously record cytoplasmic Ca^{2+} ($[Ca^{2+}]_i$) transients of intra-cellular origin in β-cells lacking direct contact. Such transients have been found to modulate β-cell rhythmicity by activating a hyper-polarizing K^+ current. Results.The transients of $[Ca^{2+}]_i$ were often synchronized in ß-cells situated up 80 µm apart. The frequency of the transients increased by 50 % when the ß-cells were exposed to 100 µmol/l of the NO donors sodium nitroprusside or hydroxylamine. Additions of gaseous NO to a final concentration of 0.1-10 µmol/l was also effective in inducing synchronized transients similar to those normally appearing in ß-cells. When introduced at a concentration of 1 mmol/l the NO donors suppressed the appearance of transients. Both an established scavenger of NO (500 µmol/l oxyhemoglobin) and an inhibitor of its production (10 mmol/l N^{ω}-nitro-L-arginine ester) reduced the number of $[Ca^{2+}]_i$ transients. Conclusion. Although toxic for the β-cells when produced in excessive amounts, gaseous NO fulfils the criteria for a molecule of islet origin acting as a diffusible synchronizer of the $[Ca^{2+}]_i$ rhythmicity.

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EXPRESSION OF A NEURONAL ISOFORM OF NITRIC OXIDE SYNTHASE IN RAT PANCREATIC B CELLS.

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A number of functional studies point to a control of pancreatic B cell function by a constitutive Nitric Oxide Synthase (cNOS) activity; however the nature and intra-islet localization of the enzyme is still matter of debate. **Aim**: Our work was aimed at identifying 1) the type of cNOS isoform involved and 2) its intra-islet cellular and sub-cellular localization. **Material and methods**: Molecular and biochemical approaches were applied to both isolated rat islets and the insulin secretory cell line INS_1. **Results**: Using Reverse transcription-PCR with primers based on the sequence of the rat cerebellar NOS (nNOS) and the rat endothelial cNOS, we only amplified the 550 bp fragment expected with the nNOS primers and sequencing revealed total homology with rat cerebellar NOS. The whole coding sequence was sequenced and revealed 5 allelic mutations in islets and an additional one in INS_1 cells leading to 99.8% homology with rat cerebellar NOS. Only 3 of these mutations result into a change in amino acid sequence. The expression of the 160 kDa nNOS protein identical to the cerebellar extract was also demonstrated by Western Blot. Immunofluorescence studies using a polyclonal nNOS antibody showed that nNOS is expressed in islets B cells and INS_1 cells and appeared to be mainly co-localized in insulin secretory granules. This was confirmed by electron microscopy which also brought evidence for the presence of nNOS, albeit to a lesser extent, in mitochondria, golgi apparatus and nucleus of B cells. **Conclusion**: We conclude that a neuronal isoform of cNOS is expressed in rat pancreatic islet B cells and the INS_1 cell line and appears to be mainly localized in secretory granules.

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NITRIC OXIDE AND CARBON MONOXIDE - A NEW CLASS OF MESSENGER MOLECULES REGULATING INSULIN SECRETION.

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Aims: Production of nitric oxide and carbon monoxide, through the action of nitric oxide synthase (NOS) and heme oxygenase (HO), respectively, has been detected in the islets of Langerhans. The inducible isoform of NOS (iNOS) is induced by cytokines and might contribute to the development of type-I diabetes. HO activity, however, is proposed to protect against the development of IDDM. In the present study we have detected and quantified NO and CO production within mouse islets of Langerhans concomitant with measuring its influence on insulin secretion. We have also tried to explain how theses two gases exert their effects and how their activities interfere.

Methods: Islet NO production was mesured by HPLC-technique and CO production was gaschromatographically determined.

Results: Depending on the concentration used, exogenously applied NO gas had mainly inhibitory but, under certain conditions, stimulatory effects on insulin secretion, while CO showed only stimulatory effects. The stimulatory, but not the inhibitory effects of NO on insulin secretion were extinguished after membrane depolarization. In contrast, the stimulatory effects of CO were not influenced by membrane depolarization, but were abolished in the presence of the guanylate cyclase inhibitor ODQ. Islet NO-production, was significantly inhibited both by CO itself and by its precursor hemin, while islet CO-production was increased by NO. Injection of lipopolysaccharide (LPS), known to generate islet cytokine production and induce the development of IDDM, increased both NO- and CO-production within the islets of Langerhans. Fasting, known to decrease insulin secretion, induced islet iNOS expression and increased NO-production, while CO-production was decreased.

Conclusion: Both NO- and CO-production seem to be involved in many different physiological and pathophysiological ("prediabetic") states in the islets of Langerhans. Our data speak in favour of NO as a negative modulator of insulin secretion, while HO-activity and CO-production seem to defend and protect the islets.

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Expression of arginase isoforms in rat islets of Langerhans

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Aims: Arginase, an enzyme present in islets of Langerhans and the beta cell line RINm5F, can be induced in macrophages by various stimuli including the Th2-derived cytokines IL-4 and IL-10. During insulitis, induction of increased flux of arginine substrate via arginase in beta cells could reduce production of cytotoxic NO. The aim of this study was to identify the arginase isoforms expressed in rat islets of Langerhans as a prelude to the investigation of arginase induction and regulation in rat and human islets. **Materials and Methods:** Islets were obtained by collagenase digestion of Wistar rat pancreata. Protein samples from sonicated islets were separated by SDS-PAGE and immunoblots prepared using chicken anti-rat arginase I, and anti-human arginase II as primary antibodies. Mouse liver (for arginase I) and mouse kidney (for arginase II) were used as reference tissues for the two distinct arginase isoforms. **Results and Conclusions:** Western blot results show that rat islets of Langerhans express both isoforms of arginase at levels comparable to those found in the mouse reference tissues. The identification of physiological and/or pharmacological inducers of the islet arginase isoforms may be of relevance to the control of NO generation and subsequent beta cell damage during insulitis.

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TOTAL PARENTERAL NUTRITION IN RATS: ISLET NITRIC OXIDE PRODUCTION, CYCLIC NUCLEOTIDES AND INSULIN RELEASE.
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Aims: To elucidate the influence of long term elevation of plasma lipids on insulin and glucagon secretion in relation to the formation of nitric oxide (NO) and cyclic nucleotides in the pancreatic islets.
Methods: Normal healthy rats were subjected to total parenteral nutrition (TPN) for 10 days. We then measured insulin and glucagon secretion as well as islet cAMP, cGMP accumulation and NO synthase (NOS) activity in response to different secretagogues. Hormones and cyclic nucleotides were determined by radioimmunoassay and NOS activity by HPLC technique.
Results: Incubation of isolated islets from TPN rats showed an increased insulin secretion accompanied by an enhanced accumulation of cAMP and cGMP at basal glucose (1 mM). Glucagon secretion, however was decreased. Glucose-stimulated (16.7 mM) insulin secretion, and islet cAMP content of TPN rats were strongly suppressed, whereas islet cGMP as well as islet NO-production were markedly increased. This increase in NO-production was largely derived from inducible NOS (iNOS) activity. Addition of glucagon-like peptide 1 (GLP-1) to control islets media at 16.7 mM glucose brought about a marked potentiation of insulin secretion and a 2-fold increase in islet cAMP accumulation, whereas it totally suppressed the glucose-stimulated cGMP elevation. These effects of GLP-1 was much greater in the islets of TPN animals. Compared to controls the phosphodiesterase inhibitor IBMX induced a greater insulin secretory response from the TPN treated animals both *in vitro* and *in vivo*.
Conclusions: The results show that long-term treatment with TPN brings about the expression of iNOS activity in the islets and a strong increase in NO-production, which might contribute to the impairment of glucose-stimulated insulin release. This impairment is, at least partly, compensated for by a marked increase in the islet cyclic AMP secretory pathway.

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THE EFFECT OF TNF-α ON CELL GROWTH AND PLC-γ EXPRESSION IN INSULIN-SECRETING β-CELLS
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Tumor necrosis factor (TNF) inhibits insulin secretion and exhibits cytotoxic effects on insulin-secreting β-cells. In this study, we have characterized the effects of TNF-α on phospholipase C-γ (PLC-γ) expression in an insulin-secreting β-cells line (β-TC3). TNF-α inhibited the cell growth of β-TC3 in a time- and dose-dependent manner (0-600 U/ml). In parallel experiments, the insulin-secreting capacity of β-TC3 cells was measured in the presence of 20mM glucose and 1mM carbachol. Results showed an initial increase in the insulin-secreting capacity after 4 h treatment with TNF-α. Prolonged TNF-α treatment (up to 48 h) resulted in a decrease in the insulin-secreting capacity. A decrease in PLC-γ expression was observed after prolonged treatment of β-TC3 cells with TNF-α in combination with LiCl (10mM). LiCl alone was essentially without effect. In addition to decreased expression levels of PLC-γ, a translocation of the enzyme from the cytosol to the cell membrane of β-TC3 cells was observed. These observations imply a dual mechanism for TNF-α on the regulation of insulin secretion in β-cells.

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Lipids and Islet Function

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LIPOTOXICITY IN ISOLATED HUMAN ISLETS.
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Aims: Prolonged exposure of rodent islets to high concentrations of free fatty acids (FFA) causes alterations of insulin release (IR) and reduced islet cell survival (lipotoxicity). We evaluated the phenomenon of lipotoxicity in isolated human islets (HI). Materials and Methods: HI were prepared by collagenase digestion and density gradient purification from four pancreases, incubated either with or without 0.25, 0.5 or 1.0 FFA (oleate-to-palmitate, 2-to-1), up to 24h, and then acutely challenged with 3.3 and 16.7 mM glucose (G), in sequence. Islet cell survival was evaluated by the MTT staining and/or the TUNEL technique. Bcl-2 (an antiapoptotic molecule) mRNA expression in the islets was assessed by RT-PCR. Results: IR (μU/ml) from control islets (Ctrl) was 181±85 (mean±SD) and 408±207 respectively at 3.3 and 16.7 mM G ($p<0.01$). Twentyfour hour exposure to 0.25 mM FFA caused a slight increase of IR at low glucose (240±42, NS vs Ctrl), and a slight decrease of IR at high glucose (310±66, NS vs Ctrl). Pre-culture with 0.5 mM FFA determined no major change of IR at 3.3 mM G (191±52, NS vs Ctrl), and a significant reduction of IR at 16.7 mM G (265±145, $p<0.05$ vs Ctrl). With 1.0 mM FFA, IR decreased significantly in response to both low (123±68, $p<0.01$ vs Ctrl) and high (88±52, $p < 0.001$ vs Ctrl) glucose.With 1.0 mM FFA, after 4h incubation a significant increase of IR at 3.3 mM G (532±92, $p<0.02$ vs Ctrl) was observed, whereas after 12h the secretion of insulin was significantly decreased at both 3.3 and 16.7 mM G, with no further significant changes at 24h incubation. In the islets cultured for 24h with 1.0 mM FFA, MTT staining and the TUNEL technique revealed a large amount of dead cells, and RT-PCR studies revealed a marked decrease of Bcl-2 mRNA expression. Conclusions: These results show that lipotoxicity occurs with isolated HI; the phenomenon can be observed with relatively low (0.5 mM) FFA concentration, and is fully manifest at higher (1.0 mM) FFA level; lipotoxicity in human islets is characterized by functional alterations (early increase of IR in response to low glucose, followed by a marked reduction of IR at both low and high glucose challenge), and citotoxicity.

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EFFECTS OF FATTY ACIDS ON ß-CELL SURVIVAL.
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Chronic exposure to elevated fatty acids (FA) levels induces a series of insulin secretory abnormalities. Aim: To investigate whether FA also affect beta cell survival we cultured rat pancreatic islets for up to 7 days with or without 2 mM oleate/palmitate (2:1) and then measured apoptosis by determining Annexin V binding. This protein has a strong affinity for phosphatidylserine, a phospholipid that during the early phase of apoptosis translocates from the inner face of the plasma membrane to the cell surface, where may bind to FITC-conjugated Annexin-V. Results: 44±7% of cells were in apoptosis in islets cultured 7 days with FA, but only 18±3% in control islets (mean±SE,n=5,$p<0.01$). Necrotic cells, identified by propidium iodide, were similar in the two groups (5.5±0.2 and 4.2±0.4%, respectively). We also studied FA effect on ß-cell replication in INS-1 cells. 48 h after implantation cells were cultured in the presence or absence of 2 mM oleate/palmitate (2:1) and the MTT test (an index of metabolically active cells) was performed 3, 5 and 7 days later. Values were similar to control cells after 3 days, but were reduced by 25±2% and 52±2% after 5 and 7 days, respectively (mean±SE,n=5, $p<0.01$).
Conclusions. These data indicate that chronic exposure to FA increases apoptosis in rat pancreatic islets, and inhibits proliferation in ß-cell in permanent culture (INS-1 cells). These effects may contribute to the ß-cell failure observed in type 2 diabetic patients.

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PROFOUND ALTERATION IN PANCREATIC ISLET ALPHA 2 ADRENOCEPTORS AFTER A 48 H TRIGLYCERIDE INFUSION IN RATS.

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We previously showed that a 48h infusion of a triglyceride emulsion (Intralipid) in rats (IL rats) induced a 2.5 fold increase in glucose-induced insulin secretion (GIIS) *in vivo*, partly due to a decrease in sympathetic nervous activity. Moreover, low concentrations of an alpha 2 agonist oxymetazoline, which were ineffective in control rats dramatically reduced GIIS in IL rats.
Aims: We investigated here the extent to which high GIIS resulting from low sympathetic nervous activity could be due to changes in alpha 2 adrenoceptors on the pancreatic ß-cell membrane.
Materials and Methods: Normal Wistar rats were infused for 48h with a triglyceride emulsion, leading to a 3 fold increase in plasma free fatty acids. At the end of infusion pancreatic islets were isolated using collagenase digestion and Ficoll gradient. Islets from control and IL rats were sonicated and the homogenate was used for binding experiments with RX821002 as a radioactive alpha 2 specific ligand. Non specific binding was determined using epinephrine.
Results: There was a sharp decrease in alpha 2 adrenoceptors number (Bmax: IL rats; 9.05 ± 1.23 fmol/mg of protein, n=3; C rats: 36.10 ± 8.01 fmol/mg of protein, n=5, $p<0.05$) and an increase in affinity (Kd = 0.23 ± 0.08 vs. 2.51 ± 0,38 nM in control rats, $p<0.01$).
Conclusion: These data indicate that lipid infusion sharply alters the main characteristics of islet alpha 2 adrenoceptors, either directly or indirectly, via the decrease in the sympathetic tone. These changes may have consequences on the intracellular signaling leading to the control of insulin secretion.

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METFORMIN PREVENTS LIPOTOXICITY IN RAT PANCREATICISLETS.

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We cultured rat pancreatic islets for 48 h with or without FA (2 mM oleate/palmitate 2:1,) in the presence or absence of metformin (24 µg/ml), and then measured insulin release, glucose utilization (formation of ^3H_2O from (5-^{3}H)-glucose) and glucose oxidation (formation of $^{14}CO_2$ from U-^{14}C-glucose) at 1.4 and 16.7 mM glucose. Results. When compared to control islets, islets exposed to FA showed, , an increased basal (185±25 vs 28±5 pg/islet/90 min, n=7, $p<0.01$) and a decreased glucose-induced insulin release (158±32 vs 646±80, $p<0.01$). In islets cultured with FA and metformin both basal and glucose-induced insulin release were significantly improved (95±15 and 439±50, respectively, $p>0.01$ vs. islets cultured with FA alone). Both glucose utilization and oxidation were altered by FA: in particular, in respect to control islets, basal glucose utilization (at glucose 1.4 mM) was increased (33±1 vs. 19±2 pmol/islet/2h n=4, $p<0.05$), and glucose oxidation at 16.7 mM glucose was decreased (46±2 vs 61±3 pmol/islet/90 min, n=4, $p<0.05$). These abnormalities were prevented by the addition of metformin (24 µg/ml): basal glucose utilization = 23±0.5 pmol/islet/2h; glucose oxidation at 16.7 mM = 60±3 pmol/islet/90 min, respectively. Glucose utilization and oxidation were unaffected by metformin in control islets. Conclusions. These data indicate that metformin prevents both glucose metabolism and glucose-induced insulin release abnormalities produced by FA in rat pancreatic islets. These effetcs may contribute to the therapeutic effects to this drug in type 2 diabetic patients.

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THE INFLUENCE OF DIETS RICH IN SATURATED FATTY ACIDS DURING AND AFTER GESTATION: STUDIES ON ISOLATED RAT ISLETS.

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Restriction of protein and energy intake during gestation results in developmental defects in the endocrine pancreas and insulin resistance. It is not known whether saturated fatty acids given to mothers during the gestational period may be detrimental to the endocrine pancreas of the offspring. The aim was to test our hypothesis that a diet rich in saturated fat given during gestation and/or during the suckling period as well as the post-weaning period suppress the β cell responsiveness in the rat offspring.
Design: Female Wistar rats were given isocaloric diets rich in carbohydrate (C, 80E%) or fat (F, 58E%, mainly saturated fatty acids) before and during gestation. The F-generation was split into five subgroups: 1 and 2 continued on diet C or F in the suckling and weaning period until week 16. Group 3 with mothers on diet C continued on the C diet during the suckling period but changed to a F diet at weaning until week 16. Group 4 with mothers on diet F continued on diet F during the suckling period but changed to diet F at weaning until week 16. For group 5 the offspring of mothers given a F diet was changed to C diet during suckling until week 16. The animals were 16 weeks old (n=8 per group). After isolation, islets were incubated overnight and then perifused. The animals had similar weight gains during the study period. The fasting p-insulin and p-triglyceride levels were higher in group 2 and 3 ($p<0.05$, respectively) whereas total cholesterol levels were similar. The basal insulin response to 2.0 mM glucose from perifused islets showed no differences between the five groups. The area under the insulin response curves (AUC) were similar in response to 11.1 and 25.0 mM glucose. The total AUCs in response to 11.1 mM glucose and 100 nM GLP-1 were not significantly different (group 1: 45±7, 2:40±6, 3: 37±5, 4: 37±4 and 5: 30±5 ng/ml*20 min, respectively). In conclusion, saturated fat rich diet given during the gestational period does not alter the islet secretory responses from perifused islets compared to carbohydrate rich diet or saturated fat rich diet given after weaning period. Whether the dietary manipulation influence the insulin sensitivity is under investigation.

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DELAYED BETA-CELL DEVELOPMENT IN SECOND-GENERATION FETUSES FROM FEMALES SUBMITTED TO PERINATAL MALNUTRITION

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Aims: We have recently shown that malnutrition during the perinatal period impairs the adaptation of the endocrine pancreas to a subsequent pregnancy, with slight glucose intolerance and no increase of endocrine mass in late pregnancy. The aim of the present study is to investigate the impact of this inadaptation on the development of the endocrine pancreas in the second generation fetuses. **Materials and methods:** Female rats were malnourished during pregnancy and until the end of lactation and the F1 female offspring, normally nourished after weaning, were mated at 8 months of age. Morphometrical measurements of insulin-positive cells were performed on pancreatic sections from E14 and E20 F2 fetuses and compared to fetuses from age-matched control dams. **Results:** Beta-cell mass was significantly decreased in E20 F2 fetuses (197±27 µg, *vs* 281±40 µg in control fetuses, $p<0.01$), as well as the number of islets/cm^2 (6±0.5 *vs* 7.4±0.4 in controls, $p<0.01$). In E14 pancreatic rudiments, the number of cells positive for glucagon only was identical in both groups. The total number of cells immunopositive for insulin was similarly identical, but 80% of these cells were also positive for glucagon in F2 fetuses from females malnourished in the perinatal period, whereas it concerned only 60% of the insulin-positive cell population in control F2 fetuses. **Conclusions:** These results suggest that the inadaptation of the maternal endocrine pancreas to pregnancy due to early malnutrition, delays beta-cell maturation and development in the second generation.

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The Role of IAPP in Islet Hormone Secretion

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IDENTIFICATION OF A MUTATION IN THE AMYLIN PROMOTER GENE

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Islet amyloid polypeptide (IAPP) or amylin is the main component of pancreatic islet amyloid found in most patients with type 2 diabetes. In man, its synthesis is controlled by a single gene located on chromosome 12, which contains three exons and two introns. Abnormalities of the IAPP gene may play a potential role in the pathogenesis of type 2 diabetes. A S20G mutation in the exon 3 of the IAPP gene has been reported in 4% of Japanese subjects with type 2 diabetes. Mutations in the promoter region of the IAPP gene could result in abnormal regulation or expression of this gene and, therefore, they also may play a role in type 2 diabetes. **Aim**: The aim of our study was to investigate the presence of mutations in the promoter region and in the exon 3 of the IAPP gene in Spanish subjects with type 2 diabetes. **Patients ands Methods**: We recruited 150 unrelated type 2 diabetic patients and 136 control subjects. Since gestational diabetes (GDM) can be considered a prediabetic state, we also screened 40 women with GDM and 36 pregnant controls. Mutations were searched by SSCP and then confirmed by direct sequencing. **Results**: We identified a heterozygous mutation in the IAPP promoter gene, consisting of a G to A substitution at position -132 from the cap-site of the gene in 12 (8%) type 2 diabetic patients and in 7 (5%) control subjects (p=0,3). The screening in pregnant women revealed the same G to A change in homozigosis in one woman with GDM, whose parents -both with type 2 DM- were heterozygous for the mutation. The screening in exon 3 did not detect the S20G mutation in any of the subjects studied. We only found a polymorphism at codon 31 (AAC/AAT) in one diabetic patient. **Conclusions**: We report the first mutation detected in the IAPP promoter gene, which was more common in diabetic patients than in control subjects, although it did not reach statistical significance. Functional studies are now in progress to evaluate the effect of this mutation on the promoter activity and to determine its role in IAPP gene transcription. On the other hand, our study suggests that IAPP gene mutations in exon 3 do not play a role in the pathogenesis of type 2 diabetes in Spanish population.

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SYNTHETIC PRO-ISLET AMYLOID POLYPEPTIDE IS CLEAVED BY RECOMBINANT PROHORMONE CONVERTASES, PC2 AND PC3 IN VITRO.

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Islet amyloid polypeptide (IAPP, amylin) is derived from a larger precursor, proIAPP, 67 amino acids in man, by proteolytic processing at the C- and N-termini. Co-localisation of insulin and IAPP in β-cell granules suggests that the prohormone convertases PC2 and PC3 which are responsible for proinsulin processing are involved. **Aims:** To determine if human proIAPP is cleaved by PC2 and PC3 in vitro. **Methods;** Human synthetic proIAPP produced by solid state synthesis or human recombinant proinsulin was incubated with recombinant PC2 or PC3 for time periods up to 36h at 37°C. Reaction products were analysed by MALDI-TOF mass spectrometry and HPLC. **Results:** Incubation of proinsulin with mature PC2 (at pH5) or PC3 (at pH6) resulted in two products of 3305Da (equivalent to C-peptide-KR) and 6125 Da (insulin-RR). PC2 in 5mM calcium pH5 cleaved proIAPP after 120m at the N-terminal basic cleavage site proIAPP $_{11,12}$ (products of 1323 and 6097Da) and subsequently at proIAPP 51,52 at the C-terminal. Mature PC3 (86kDa) incubated with proIAPP at pH 6 resulted in products of 1868 and 5553Da from an initial cleavage at proIAPP$_{51,52}$. Later activity at the N-terminal site resulted in proIAPP $_{12-51}$ (equivalent to IAPP) and the N-terminal fragment of 1323Da. The presence of the intermediates was confirmed by HPLC. When both enzymes were present together, all products were identified in the incubation mixture. Incubation of proinsulin and proIAPP with PC3 did not result in any apparent competition for substrate **Conclusions**: These data suggest that proIAPP is processed sequentially in maturing β-cell granules- by PC3 at the C-terminal junction followed by PC2 at the N-terminal at a lower pH. In Type 2 diabetes increased secretion of proinsulin could be accompanied by proIAPP.

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INSULIN AND ISLET AMYLOID POLYPEPTIDE 'AMYLIN' FORM STABLE MOLECULAR COMPLEXES IN VITRO AFFECTING FIBRIL FORMATION

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Islet amyloid polypeptide (IAPP) is co-stored in β-cells and co-secreted with insulin. In Type 2 diabetes, secreted IAPP aggregates to form islet amyloid deposits which have a role in the progressive deterioration of insulin secretion. The factors that promote conversion of soluble human IAPP (hIAPP) into insoluble amyloid fibrils are largely unknown; interaction of IAPP with β-cell granule components could influence fibrillogenesis. **Aims:** To examine molecular interactions between IAPP and insulin. **Methods**: Formation of complexes in mixtures of synthetic hIAPP and human insulin peptides was examined by Western blotting, circular dichroic spectroscopy (CD) and nanoflow electrospray interface mass spectroscopy (ESIMS). **Results**: Interaction between insulin and hIAPP was shown by immunoprecipitation from mixtures of hIAPP and insulin, gel electrophoresis and Western blotting with specfic antisera to IAPP or insulin. Mass analysis of peptides by ESIMS in an insulin/IAPP mixture (100ug/ml) incubated for up to 24h showed the expected masses of insulin monomers (5806.49+/-0.09Da) and hIAPP monmers (3902.88+/-0.41Da) as well as a 1:1 complex of insulin and IAPP (9709.13+/-0.08Da). Mixtures of hIAPP and human insulin B chain demonstrated strong intermolecular interactions with formation of multimers of different ratios of hIAPP and B-chain. Incubation of hIAPP with insulin delayed conversion of hIAPP to β-sheet conformation examined by CD;insulin and B-chain inhibited the formation of IAPP fibrils. **Conclusions**: hIAPP forms complexes with insulin which could stabilise IAPP in the insulin granule *in vivo* and influence the potential of IAPP to form extracellular amyloid fibrils in diabetes.

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AMYLOID FIBRILS ARE FORMED FROM PROISLET AMYLOID POLYPEPTIDE IN TRANSGENIC MOUSE INSULINOMA CELLS.

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Islet amyloid is formed from islet amyloid polypeptide (IAPP) in type 2 diabetes and in transgenic mice expressing the gene for human IAPP (hIAPP); putative factors promoting fibril formation include aberrant processing and/or overproduction of proIAPP/IAPP. **Aims**: To examine the causative factors for fibril formation in mouse cells expressing the hIAPP gene. **Methods**: Mice expressing SV40 large T antigen linked to insulin gene promoter were cross bred with transgenic mice expressing hIAPP gene. Offspring carrying both transgenes were identified by Southern blotting and islet cell tumours removed from mice (aged 12-14 weeks). Tumour cells cultured in dishes and on filters were analysed for the presence of insulin, IAPP and fibrils by immunolabelling for light and electron microscopy (em). Insulin and IAPP secretion was determined by by RIA. **Results:** Transgenic mouse tumours consisted of well granulated β-cells when visualised by em. Secretion from isolated cells cultured at 4.2mM glucose was 2388 and 3084 pmol/l/24hours insulin or IAPP respectively and was not significantly increased by culture in 16mM glucose. Immunoreactivity for IAPP (IAPP-ir) was present in β-cell granules and lysosomes. Extracellular amyloid fibrils were were present at 7 days culture at 11mM glucose and were IAPP-ir for human proIAPP peptides (antisera to IAPP$_{1-37}$, C-and N-terminal peptides of proIAPP); fibrils were located between cells and at the margin of cell clusters. Fibrils formed between the cells and filter matrix suggesting inadequate clearance of secreted hIAPP. Intracellular fibrils which were proIAPP-ir were present in β-cells undergoing apoptosis or necrosis. **Conclusions;** Incompletely processed proIAPP and reduced peptide clearance could promote fibrillogenesis of IAPP and contribute to intracellular amyloid formation in dying cells.

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FAILURE OF AMYLIN TO DIRECTLY AFFECT GLUCAGON RELEASE. STUDY IN THE PERFUSED RAT PANCREAS.

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Aims: Administration of pramlintide, a human amylin analogue, has been shown to reduce postprandial hyperglycaemia in diabetic subjects. Besides slowing gastric emptying, pramlintide induces a reduction of plasma glucagon levels. To gain further insight into this aspect, we have examined the direct effect of rat amylin on glucagon secretion in the rat pancreas. **Materials and Methods:** The study was performed in the isolated perfused rat pancreas. Fed male Wistar rats were used as donors. Perfusate consisted of Krebs-Henseleit buffer supplemented with dextran (4%), albumin (0.5%) and glucose (3.2, 5.5 or 11 mM). **Results:** At 5.5 mmol/l glucose, infusion of 1 nmol/l rat amylin did not modify either unstimulated glucagon output ($F_{10,50}$=1.08; n.s.) or the glucagon responses elicited by arginine (5 mM) (incremental area:2.2±0.4 vs. 2.3±0.7 ng/20 min in control experiments; p=0.9), and VIP (1 nM) (3.5±1 vs. 3.9±1.3 ng/20 min; in controls p=0.78). Amylin also failed to affect the glucagon response induced by lowering perfusate glucose concentration from 11 to 3.2 mM (4.5±0.3 vs. 4.8±1 ng/20 min in controls; p=0.8), and the glucagon suppression evoked by an increase in glucose from 3.2 to 7 mmol/l (decremental area: 2.4±0.8 vs. 2.6±0.4 ng/20 min in controls; p=0.7). **Conclusions:** In the perfused rat pancreas, infusion of amylin, at a concentration one order of magnitude higher than that reported to exert insulinostatic activity, failed to modify glucagon release. Thus, the described reduction of circulating glucagon induced by amylin should be interpreted as an interference of this peptide with a humoral/neural stimulatory signal reaching the alpha-cell.

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APOLIPOPROTEIN E GENOTYPE IS NOT ASSOCIATED WITH ISLET AMYLOID AS ASSESSED BY DISEASE SEVERITY IN TYPE 2 DIABETES.

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Apolipoprotein E (ApoE) is a component of all forms of amyloid deposition, including cerebral and pancreatic islet amyloid and has been postulated to have a role in amyloid fibril formation. ApoE genotype is an important marker for Alzheimer's disease (AD); ApoE ε4 allele confers the highest risk for early onset, degree of severity and amyloidosis in AD. ApoE genotype is not linked to Type 2 diabetes but its relationship to islet amyloidosis is unkown. **Aims**: To examine the association of ApoE genotype with disease severity in diabetes clinic patients diagnosed >40y and with islet amyloid in post-mortem pancreas from diabetic subjects. **Patients and Methods**: Since more extensive islet amyloidosis is associated with severity of disease, two clinic patient groups were examined; severe diabetes (S) requiring insulin within 6y (n=166) and mild diabetes (M) on monotherapy and sub-maximal doses of sulphonylureas at 10y from diagnosis (n=142). Patients with known lipid dyscrasias were excluded. DNA extracted from clinic blood samples and from 101 non-diabetic subjects (C) was genotyped for ApoE. **Results:** There were no significant differences between allelic frequencies between the groups; ε2, 0.06(S), 0.10(M), 0.07(C) (n=20,29,13 respectively); ε3, 0.85(S), 0.77(M), 0.81(C) (n= 281,219,163 respectively); ε4, 0.09(S), 0.13(M), 0.12(C) (n= 31,36,26 respectively) and there was no significant deviation from Hardy-Weinberg equilibrium. DNA extracted from wax embedded post-mortem pancreas from 10 diabetic subjects, 7 with varying degrees of islet amyloid was genotyped for ApoE. Severe amyloid was present in 4/5 specimens of ε3/4 genotype, moderate amyloid in 2/4 of the ε3/3 specimens and moderate deposits in the one ε2/3 subject. **Conclusions:** ApoE genotype is unlikely to be related to severity of type 2 diabetes as assessed by therapy requirements.

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Modulation of Insulin Secretion

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ISLET ADAPTATION TO DEXAMETHASONE-INDUCED INSULIN RESISTANCE IN RATS: ROLE OF ISLET NERVES

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Aims: The mechanism of hyperinsulinemia during corticosteroid induced insulin resistance is not known. We examined islets from dexamethasone (DEX)-treated rats with respect to insulin secretion and the possible contribution of islet nervs to the islet adaptation to insulin resistance. **Materials and Methods:** Islets (freshly isolated or cultured overnight) from control and DEX-treated (2mg/kg body wt., i.p. daily for 12 days) Sprague-Dawley rats were used. Neural immunoreactivity for tyrosine hydroxylase (TH; marker for adrenergic nerves) or Vasoactive intestinal polypeptide (VIP; marker for cholinergic nerves) were determined in sections of isolated islets. **Results:** Plasma insulin levels were 653±105 pmol/l after DEX compared to 183±49 pmol/l in controls (P<0.004; n=14). Plasma glucose levels did not differ between groups (P<0.09). The insulin response to 8.3 mmol/l glucose was increased 4-fold in freshly isolated perifused islets from DEX-treated rats compared to controls (14±2.3 vs. 3.2±1.4 pmol/islet per min after 10 min; P<0.001). Insulin content (8.8±1.7 vs. 9.1±0.7 pmol/islet P=0.85) or the number of nerve terminals did not differ between DEX- and control islets. Incubation experiments revealed a marked left-ward shift (P<0.05) of the glucose dose-response curve in freshly isolated islets from DEX-treated rats with no difference at zero or 20 mmol/l glucose (maximal stimulation; n=11-18). Overnight islet culture resulted in a diminished insulin response to glucose (8.3-20 mmol/l) in DEX compared to controls (227±40 vs. 465±71 pmol/islet per 60 min at 20 mmol/l glucose; P<0.006) and a 50% reduction in VIP- and TH-immunoreactive nerve terminals in both DEX and control islets compared to freshly isolated islets. Five-day islet culture with DEX (100 nM) did not affect the insulin response to a subsequent challenge with glucose (1.8-20 mmol/l) compared to controls. **Conclusions**: DEX-induced insulin resistance in rats leads to enhanced glucose-stimulated insulin secretion from isolated islets by a mechanism that is not a direct action of DEX on the islet B-cells but rather an indirect adaptive mechanism involving islet nerves.

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INVOLVEMENT OF A GLYCINE RECEPTOR IN GLYCINE-INDUCED INSULIN RELEASE FROM PERFUSED RAT PANCREAS

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Glycine acts as a neurotransmitter by activating strychnine-sensitive receptor chloride channels in the central nervous system. In the pancreas, the stimulatory action of glycine on insulin secretion is attributed to depolarisation of the membrane through its co-transport with Na^+ but, recently, glycine receptors have been reported to be expressed in islets. This study was designed to investigate whether glycine receptors are involved in the insulin secretory action of the amino acid in the isolated perfused pancreas of the rat. In pancreata perfused with 8.3 mmol/l glucose, glycine (1-30 mmol/l) induced a biphasic and concentration-dependent insulin response with a maximum peak of +1150% at 2 min. However, in the presence of the inhibitor of glycine transport sarcosine (10 mmol/l), glycine (0.3-10 mmol/l) provoked a concentration-dependent insulin response only in a peak shape with a maximum of +250% at 2 min (half-maximal response at 2.5 ± 0.2 mmol/l). The stimulatory action of glycine (3mmol/l) was prevented by the glycine receptor antagonist strychnine (30 µmol/l) and reduced (inhibition of 60% at 2 min, p<0.001) after pretreament with the chloride uptake inhibitor bumetanide (10 µmol/l). In the presence of 8.3 mmol/l glucose, the agonist of glycine receptors, β-alanine (1-30 mmol/l) also elicited a transient and concentration-dependent insulin response which was strychnine-sensitive. These results suggest that in addition to the uptake of glycine, a glycine receptor chloride channel is involved in the insulin secreting effect of the amino acid in the rat pancreas.

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INHIBITION OF INSULIN RELEASE BY VIP AND L-DOPA

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Aims: Mouse islets decarboxylate L-DOPA, the resulting dopamine inhibiting insulin release. Some ß-cells express tyrosine hydroxylase (TH), an enzyme in the catecholamine biosynthesis pathway that converts tyrosine to L-DOPA. However, the role of TH in islet cells is unclear. We have previously observed increased expression of TH in islets cultured with vasoactive intestinal polypeptide (VIP). The present study aimed at testing for inhibitory effects of VIP and L-DOPA on insulin release. **Materials and Methods:** BALB/c mouse islets, cultured for 4 days with or without 10 nM VIP, were preincubated at 4 mM glucose with or without 2 mM L-DOPA for 60 min, followed by 30 min at 3 mM glucose alone. They were then incubated at 20 mM glucose with or without 2 mM L-DOPA. Insulin was radioimmunoassayed. **Results:** At 3 mM glucose, all islet groups which had first been exposed to VIP or L-DOPA showed a decreased basal insulin secretory response (VIP/Ctrl 0.10±0.02, Ctrl/L-DOPA 0.09±0.02, VIP/L-DOPA 0.10±0.02 ng insulin/h per µg dry weight; mean±SEM) as compared to controls (Ctrl/Ctrl 0.13±0.03; ANOVA: $P<0.03$). When comparing islets cultured with and without VIP, those cultured with VIP showed a significantly lower insulin secretory response to 20 mM glucose (4.36±0.30) than islets not exposed to VIP (6.51±1.02, Fischer's PLSD $P<0.001$). Preincubation with L-DOPA diminished the subsequent secretory response to 20 mM glucose, whether in the presence or absence of L-DOPA (*t*-test: $P<0.001$). **Conclusions:** Pretreatment with either VIP or L-DOPA inhibits the subsequent insulin release. As VIP enhances TH expression in islet cells, the secretory inhibition may reflect enhanced formation of endogenous L-DOPA and dopamine.

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N-TERMINAL GLYCATION OF CCK-8 ABOLISHES ITS INSULINOTROPIC ACTION ON CLONAL B-CELLS

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Glycation of CCK-8 protects against aminopeptidase degradation and substantially enhances the potency of the peptide as an anorectic agent. Insulin-releasing activity of this novel analogue was evaluated using glycated CCK-8 prepared under hyperglycaemic reducing conditions and purified by RP-HPLC. Electrospray ionisation mass spectrometry and automated Edman degradation demonstrated that CCK-8 (1305.3 Da) was glycated specifically at the amino terminal Asp^1 residue. Effects of Asp^1-glucitol CCK-8 and CCK-8 were examined using glucose-responsive clonal BRIN-BD11 cells. In acute (20 min) incubations, 10^{-10} mol/l CCK-8 enhanced insulin release by 1.2-1.5-fold at 5.6-11.1 mmol/l glucose ($P<0.001$). The stimulatory effect induced by 10^{-10} mol/l CCK-8 was decreased by 21% following glycation ($P<0.001$). At 5.6 mmol/l glucose, CCK-8 at concentrations ranging from 10^{-11} to 10^{-7} mol/l induced a significant 1.6-1.9 fold increase in insulin secretion ($P<0.01$). The effect of Asp^1-glucitol CCK-8 over the entire range 10^{-11} to 10^{-7} mol/l was decreased by 21-35% ($P<0.001$) compared with CCK-8, and its insulinotropic action was effectively abolished. Asp^1-glucitol CCK-8 at 10^{-8} mol/l also completely blocked the stimulatory effects of 10^{-11} to 10^{-8} mol/l CCK-8. These data indicate that substitution of a glucitol adduct at the amino-terminal Asp^1 residue increases in vivo satiating effects but effectively abolishes and/or antagonises the insulinotropic activity of CCK-8.

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THE CYCLIC AMP SIGNALLING PATHWAY RESTRAINS ISLET CHOLECYSTOKININ-INDUCED PHOSPHOLIPASE A_2 ACTIVATION: A NOVEL MODULATORY REGULATION

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Aim: Cholecystokinin (CCK) induces insulin secretion through stimulation of phospholipase C (PLC) and, as we recently have shown, Ca^{2+}-dependent and Ca^{2+}-independent phospholipase A_2 (PLA_2). Now, our aim was to characterise CCK-induced PLA_2 activation in pancreatic islets with regard to the possible existence of a cross-talk between CCK-induced PLA_2 stimulation and the cyclic AMP (cAMP)-protein kinase A (PKA) signalling pathway. **Method:** We measured efflux of radioactivity from normal rat islets incubated (5.6 mM glucose) in the presence of tritiated AA, which is a method known to reflect PLA_2 activity. **Results:** The adenylate cyclase activator forskolin (1 µM), the phosphodiesterase inhibitor IBMX (0.1 mM) and the cAMP analogue dbcAMP (1 mM) reduced CCK-8 (the C-terminal octapeptide of CCK; 100 nM)-induced efflux of [^{3}H]AA by 57±10% ($p>0.001$), 61±18% ($p=0.006$) and 50±19% ($p=0.025$), respectively. In contrast, [^{3}H]AA efflux induced by the cholinergic agonist carbachol (100 µM), which also activates PLA_2, was unaffected by forskolin, IBMX and dbcAMP. To confirm that the interaction induced by the cAMP-PKA pathway involves PLA_2, we examined the impact of forskolin, IBMX and dbcAMP on [^{3}H]AA efflux induced by the PLA_2 activator mellitin (2 µg/ml), and found that the efflux was decreased by 33±10% ($p=0.008$), 46±12% ($p=0.001$) and 46±10% ($p=0.001$), respectively. Furthermore, we found that the cAMP-PKA pathway-activating secretagogues glucagon-like peptide-1 (GLP-1; 100 nM), gastric inhibitory polypeptide (GIP; 100 nM) and vasoactive intestinal polypeptide (VIP; 100 nM) diminished CCK-8-induced [^{3}H]AA efflux by 44±14% ($p=0.009$), 63±25% ($p=0.025$) and 60±19% ($p=0.006$), respectively. **Conclusions:** Our results show that the cAMP-PKA pathway has a restraining effect on PLA_2-mediated islet action of CCK, indicating that the cAMP-PKA pathway exerts a dual modulatory impact on β-cell function. Furthermore, since this restraint is absent during carbachol-induced PLA_2 activation, our results also show that CCK and carbachol stimulate PLA_2 by different mechanisms, or activate different PLA_2 subtypes.

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Dissociation of cAMP generation and insulinotropic effects of PACAP38 in diabetic GK rat islets

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The impaired glucose-induced insulin release in isolated pancreatic islets of GK rat is restored through exaggerated forskolin-induced cAMP generation. Here we tested the effect of pituitary adenylate cyclase-activating polypetide (PACAP) which is generally suggested to exert its insulinotropic effects through stimulation of adenylyl cyclase (AC) and cAMP generation. The effects of PACAP38 at 3.3 and 16.7 mM glucose were studied on concurrent insulin release and cAMP generation from islets isolated from normal and diabetic GK rats. As expected, insulin release was significantly decreased in GK islets in response to 3.3 mM ($p<0.03$ *vs.* controls) or 16.7 mM glucose ($p<0.02$ *vs.* controls). In normal islets, 100 nM PACAP did not increase insulin release at 3.3 mM glucose but significantly enhanced the responses at 16.7 mM glucose ($p<0.008$ with PACAP *vs.* without stimulation). PACAP, however, failed to enhance concurrent cAMP generation at either glucose concentration in normal islets. A contrasting picture was noted in GK islets, where PACAP stimulation failed to enhance insulin release at 3.3 or 16.7 mM glucose while concurrently eliciting a ~5 fold exaggerated cAMP generation at either glucose concentration from the diabetic islets ($p<0.03$ with PACAP *vs.* basal values). This exaggerated cAMP generation in GK islets was induced with 10 or 100 nM PACAP. In conclusion, the insulinotropic effects of PACAP in normal islets were elicited in the absence of concurrent cAMP generation. By contrast PACAP induced exaggerated cAMP generation in GK islets that was not accompanied by insulin release. These findings are first evidence that exaggerated islet cAMP generation may not necessarily lead to induction of insulin release at least in diabetic rats.

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MECHANISMS FOR GLUCOSE TOXICITY AND ITS PREVENTION BY AN INSULINOTROPIC PEPTIDE.

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Aims: Glucose toxicity in islet β-cells is implicated in the pathogenesis of diabetes. However, the signalling routs of glucose that lead to toxicity are yet to be elucidated. The present study examined whether the metabolism of glucose, a K_{ATP} closure, an increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$), or the glucosamine pathway is involved in the induction of glucose toxicity, and whether pituitary adenylate cyclase activating polypeptide (PACAP) prevents glucose toxicity. **Materials and Methods:** Isolated single β-cells from Wistar rats were cultured in control (5.6 mM) and high glucose (22 mM) conditions. After culture for 3 days, glucose toxicity was assessed by metabolic, $[Ca^{2+}]_i$ and exocytotic responses to 8.3 mM glucose, as determined by NAD(P)H, fura-2 and quinacrine fluorescence measurements, respectively. **Result:** Percentage of β-cells responding to 8.3 mM glucose with $[Ca^{2+}]_i$ increases (glucose response) was 82% in control and significantly reduced (40%) by culture with high glucose ($p<0.0001$). A K_{ATP} opener, diazoxide, added in culture increased the glucose response to 60%. Azaserine, an inhibitor of the rate-limiting enzyme of hexosamine pathway, also partly counteracted the glucose toxicity. Glucosamine and a K_{ATP} blocker, tolbutamide, mimicked high glucose in inducing toxicity. The impaired glucose response induced by high glucose (40%) and tolbutamide (35%), but not glucosamine, was significantly restored (70%, 78%) by the presence of 10^{-9} M PACAP-38 in the culture ($p<0.05$). Both NAD(P)H and quinacrine responses to 8.3 mM glucose were markedly decreased by high glucose and restored by PACAP added in culture. **Conclusions:** Glucose toxicity in β-cells is produced via closure of K_{ATP} and stimulation of glucosamine pathway. PACAP protects β-cells against glucose toxicity by counteracting the pathway mediated by K_{ATP} closure and $[Ca^{2+}]_i$ increase, but not the glucosamine pathway.

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Gene Expression in Islets

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INSULIN IS EXPRESSED IN SUBMANDIBULAR SALIVARY GLAND FROM NORMAL AND DIABETIC RATS

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The salivary glands of mammals appear to produce several biologically active peptides. We have previously reported the presence of an insulin-like immunoreactive (ILI) material in rat submandibular salivary gland (SSG). In addition, we also found that this ILI material was increased in SSG from streptozotocin (STZ) diabetic rats. **Aim** : In the present study, our aim was to search the presence of insulin mRNA in rat SSG and to compare its expression in normal and diabetic rats. **Materials and Methods** : For this purpose our experiments were performed on normal rats and STZ (66 mg/kg i.p.) diabetic rats. After SSG removal from these rats, total RNA isolation was followed by a reverse transcription (RT) using universal oligo(dT) primers. The cDNA obtained were specifically amplified by nested PCR using rat preproinsulin I and II primers. The nested PCR products were characterized by restriction enzyme digestion and they were also sequenced in both directions using the same primers as for nested PCR. Pancreas and muscle were used as control tissues. **Results** : In these conditions, we obtained for SSG, from both normal and diabetic rats, the expected 328 bp cDNA fragments corresponding to rat preproinsulin I and II coding sequences, which could be confirmed by direct sequencing. In addition, the signals of PCR products for preproinsulin I and II were found stronger for SSG from diabetic versus normal rats. **Conclusion** : Our data bring evidence 1) that preproinsulin I and II are expressed in rat SSG and 2) suggest that the increased expression found for diabetic rat might afford to play a local compensatory role in response to the drastic insulin deficiency induced by STZ.

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REGULATED PROINSULIN GENE EXPRESSION AND MATURE INSULIN SECRETION BY GH3 CELLS STABLY TRANSFECTED WITH A FURIN-CLEAVABLE HUMAN PROINSULIN cDNA

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Aims: To bioengineer regulated insulin secretion in pituitary cells.
Materials and Methods: Prolactin- and growth hormone-secreting GH3 cells were stably transfected with a furin-cleavable human proinsulin cDNA linked to the PRL promoter. Three positive clones (Ins-GH3) were subcloned by limiting dilution and characterized with regard to regulated (pro)insulin-like release and proinsulin transgene expression.
Results: Ins-GH3 clones were heterogeneous amongst them in quantitative terms of insulin production, but mature insulin secretion was obtained by all of them accounting for by 38-49% of total released (pro)insulin-like products. Insulin immunoreactivity had a granular cytoplasmatic pattern that colocalized with secretogranin (SGII) immunoreactivity. Basal and stimulated insulin release differed in the three clones, in parallel with a different expression of the proinsulin transgene. Stimulation with forskolin (FSK) elicited increased insulin release by all clones, indicating that insulin was also targeted to the regulated secretory pathway of secretion. A significant secretory response to Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) and to Tireotrophin Releasing Hormone (TRH) was also observed. FSK, PACAP, and TRH, also stimulated proinsulin transgene expression, and FSK in a dose-dependent fashion. GH3 cells, transiently cotransfected with the luciferase reporter gene placed under the control of the PRL promoter and with the glucose insulinotrophic peptide (GIP)-Receptor cDNA, showed a dose-dependent induction of luciferase activity in response to GIP.
Conclusions: these data support the hypothesis that incretin-regulated insulin gene expression, and possibly release, might be achieved in pituitary cells.

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GENE EXPRESSION MEASUREMENTS IN HUMAN ISLETS OF LANGERHANS.

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The restricted availability of human islets places limitations on the quantity of mRNA which can be extracted for use in gene expression studies (1,000 islets >0.5µg of mRNA). **Aims:** To evaluate the reproducibility of a semi-quantitative linear-PCR reaction, combined with a hybridisation technique, for measuring the relative expression of known genes from a limited quantity of islet mRNA. **Materials and Methods:** A set of 48 genes of interest were selected and cDNA fragments (200-500bp) amplified by reverse transcriptase-PCR. The PCR products were purified and immobilised onto nylon membranes. A linear-PCR reaction using an oligo(dT) primer was applied in order to generate a complex [^{32}P]-labelled probe, from human islet mRNA (<100ng), equivalent to multiple single-stranded cDNA copies of the islet mRNA pool. Labelled [^{32}P]-PCR products were purified and used as probes in hybridisation reactions with immobilised cDNA fragments on nylon membranes. **Results:** Hybridisation images were analysed by PhosphorImager and evaluated in ImageQuant. The intensity of each gene was expressed relative to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase. The hybridisation intensities (arbitary values) measured for 48 genes were compared to quantify reproducibility. The coefficient of variation for gene expression for three human islet preparations was: <25% for 33 genes; 25-50% for 10 genes; 50-100% for 5 genes. **Conclusions:** A semi-quantitative technique is established which can be used to measure the relative expression of islet genes, and which requires a limited amount of mRNA to generate a probe. The technique has the potential to determine at least 2 fold changes in islet gene expression.

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IN SEARCH OF DIABETOGENES BY DIFFERENTIAL DISPLAY OF MESSENGER RNA FROM HUMAN ISLETS OF LANGERHANS.

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Aims: Patients affected by type 2 diabetes mellitus exhibit peripheral insulin resistance and defective insulin secretion by pancreatic β cells. We decided to search for novel potential diabetogenes in islets of Langerhans from a diabetic patient who died of cerebral hemorrhage and was eligible for multiple organ donations.
Materials and Methods: Total RNA was extracted from human pancreatic islets from 1 patient affected by Type 2 diabetes mellitus and 2 normal individuals. 3 separated Differential Display experiments were performed by comparing mRNAs extracted from islets of a diabetic patient with mRNAs of normal islets.
Results: By comparing mRNAs isolated from human islets of the diabetic patient with normal islets mRNAs, we found 22 DNA fragments that are differentially expressed which were identical or had high homology with sequences retrieved by NCBI BLAST search. By Southern blotting experiments we demonstrated that 12 of these genes weren't differentially expressed, 4 genes were overexpressed in diabetic islets and 6 were overexpressed in normal islets. 2 of the genes overexpressed in diabetic islets encoded for the exocrine enzymes elastase and lisophospholipase. The remaining two genes encoded for polylg receptor transmembrane secretory component and complement C3 component. The 6 genes overexpressed in normal islets were: mitochondrial coxII, HMG-Y, human chromosome 16 BAC clone, HPH2, human Hexabrachion and prepro-alpha 1 collagen. Human polyhomeotic homolog 2 (HPH2) is a human homolog of a drosophila Polycomb-group (PcG) gene, whose precise function is at present unknown. *In situ* hybridization and immunocytochemistry experiments demonstrate that HPH2 is overexpressed in β-cells as compared with exocrine pancreas, and that it is not present in α-cells. HPH-2 expression is downregulated by 120 hours serum deprivation and upregulated by incubation with 22 mM glucose and 100 nM insulin for 48 hours in βTC-3 cells, an insulin secreting cell line.
Conclusions: Our data show that Differential Display can be applied to the study of gene expression in human islets of Langerhans and might be a powerful tool to detect novel genes involved in beta cell function and survival.

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CHROMOSOMAL ALTERATIONS IN HUMAN ISLET CELL TUMORS

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Aims: Understanding of the malignant transformation of human islet cell tumors would be useful for the development of human beta cell lines. Here we have approached the problem through the analysis of chromosomal changes in human endocrine pancreatic tumors. **Materials and methods:** 25 paraffin-embedded tumors from 23 patients were studied. In immunohistochemical stainings, all of the tumors showed positive staining for neuroendocrine markers and for insulin, glucagon, or somatostatin. High molecular weight DNA was extracted from the paraffin blocks. In some cases, the DNA had to be amplified by PCR. The comparative genomic hybridization (CGH) technique was used to evaluate DNA sequence copy number changes based on the simultaneous hybridization of differentially labelled tumor and normal DNA on normal chromosome metaphase spreads. Green to red fluorescence ratios were calculated, and a map of gains and losses through the entire genome was drawn. **Results:** DNA copy number changes were observed in 22 out of 25 tumors. The mean was 8.1 aberrations per tumor (range 1-15). The most recurrent change involved gains at chromosome 7 (in 68% of tumors). The minimal overlapping region of the gains was at 7q11.2. Other frequent gains included chromosomes 19 (60%) and 14 (56%). Chromosome 20q was amplified in 48% of the cases with the minimal overlapping region of 20q11.1-13.1. The two most frequent losses were found at 11q21-22 in 32% and at 11p13-15 in 24% of the cases. **Conclusions:** The amplified chromosomal regions contain several candidate genes which may be involved in islet cell tumorgenesis. The regions with most frequent losses are likely to contain tumor suppressor genes.

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GENE TRANSFER TO PANCREATIC ISLETS MEDIATED BY A REPLICATION INCOMPETENT HSV-1

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Aims: To examine the feasibility of gene transfer to bovine islets, using a replication incompetente HSV-1 vector encoding E.Coli-B-galactosidase (HSV-1LacZ vector) as a repoter gene, and the possible interaction with the endocrine function **Material and methods:** Bovine islets were isolated by enzymatic and mechanical digestion, and then purified by density gradient purification with Histopaque, and cultured in standard medium+bovine serum and antibiotics. A plasmid containing the expression cassette for the murine B7.1 gene and the E Coli B-galactosidase gene has been constructed by conventional recombinant DNA techniques. Islets were infected with HSV-1LacZ vector at different virus-to-target cell ratios (MOI 3-5-10). Gene transfer was assessed by direct histochemical staining for B-galactosidase protein after 2,7,12 and 14 days. Uninfected islets were used as negative controls. Islets viability was assessed before and after transfection by morphology, optical microscopy, and by evaluation of insulin release during static incubation with normal and high glucose concentration 2 days after isolation and 2-7-14 days after infection. **Results:** Best infection rate was obtained at MOI 5. Infection rate was higher 2 days after infection (50-70% of the peripheral cells), at 7-12-14 days after infection the number of stained cells per islet was moderatly lower. B-galactosidase activity was not detectable in control islets. The morphology of the islets was not different between infected and non infected islets. The in-vitro response to different glucose stimuli of infected islets was super-imposable to control islets in all experimental time points. **Conclusion:** HSV-1 can efficently infect intact islets while preserving their cellular function and could allows insertion of relevant signals into non-dividing cells.

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DIFFERENTIAL EFFECT OF SECRETAGOGUES ON ADHESION RECEPTORS OF RAT ISLET B-CELLS

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Cell-cell adhesion molecules (CAMs) and integrins are expressed at the surface of islet B-cells, and insulin secretagogues, including glucose and IBMX, increase cell-cell and cell-matrix adhesion. **Aims**: Determine whether secretagogues influence CAM and integrin expression on B-cells. **Methods**: Purified rat B-cells were incubated for 20 h under increasing concentrations of glucose ± 0.5 mM IBMX, and examined by immunofluoresecence using antibodies against different CAMs and integrins. **Results**: C-CAM, a calcium-independent CAM, was expressed on 34.7 ± 6.3% B-cells at 5.6 mM glucose, with no significant change at 16.7 mM glucose (26.0 ± 5.8%) with or without IBMX. At 2.8 mM glucose, only 7.2 % of B-cells showed a strong labeling for E-cadherin. This value increased to 21, 31 and 87% at 11.1, 22.2, and 22.2 mM glucose+IBMX, respectively. When compared to 2.8 mM glucose, flow cytometric analysis showed that the mean intensity of labeling for E-cadherin was increased by 42 ± 5, 49 ± 9 and 108 ± 14% in the presence of 11.1, 22.2 and 22.2 mM glucose+IBMX, respectively. Virtually all B-cells expressed the α3 integrin subunit at their surface. Level of labeling was homogeneous between cells and was not modified by secretagogues. However, expression of the α6 subunit (as α6β1) was heterogeneous, and labeling was increased by secretagogues. Thus, 2%, 32% and 69% of B-cells showed strong α6-labeling when incubated with 5.6, 22.2, and 22.2 mM glucose+IBMX, respectively. When compared to 5.6 mM glucose, mean intensity of fluorescence labeling was increased by 15% and 84% in the presence of 22.2 mM glucose without or with IBMX, respectively. In addition, double immunofluorescence revealed that E-cadherin and α6β1 integrin were not systematically coexpressed at the surface of B-cells, regardless of culture conditions. **Conclusions**: These experiments suggest differential regulation of adhesive molecules at the surface of B-cells, and that E-cadherin and α6 integrin, but not C-CAM and α3 integrin, may play a role in the altered adhesive properties of B-cells following exposure to secretagogues in vitro.

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ROSIGLITAZONE, BUT NOT METFORMIN OR GLIBENCLAMIDE, IMPROVES GLYCAEMIC CONTROL AND INCREASES ISLET INSULIN CONTENT.

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Aims: To compare the effects of the PPARγ agonist, rosiglitazone (RSG), metformin and glibenclamide (Glib.) on glycaemic control and pancreatic β-cell insulin content in the C57Bl/KsJ *db/db* diabetic mouse. **Materials and Methods:** C57Bl/KsJ *db/db* diabetic mice were allocated into groups (n=7) and RSG, glibenclamide or metformin were dosed admixed in the diet. Blood glucose (mmol/l) was determined after 7 and 28 days of treatment, whilst pancreatic (ng/mg of tissue) and plasma insulin (ng/ml) concentrations were determined at 28 days. A group of non-diabetic lean littermates was included for comparison. Data are presented as mean ± SD. **Results:** After 7 days of treatment RSG significantly reduced blood glucose ($p<0.001$), compared to untreated *db/db* mice and after 28 days blood glucose was similar to that of lean littermates. Neither glibenclamide nor metformin improved glycaemic control; indeed at the end of the study glibenclamide significantly worsened the hyperglycaemia ($p<0.05$).

Day	*db/db* control	RSG 1.42 mg/kg	Glib. 49.4 mg/kg	Metformin 100 mg/kg	*db/+* lean control
7	19.02±4.47	10.51±2.67	22.49±4.36	22.40±2.98	6.58±0.39
28	21.08±7.50	7.55±2.49	29.46±4.37	25.93±4.26	6.19±1.67

RSG significantly increased plasma and pancreatic ($p<0.001$) insulin concentrations, whilst glibenclamide and metformin had no effect.

Tissue	*db/db* control	RSG	Glib.	Metformin	lean control
Plasma	1.37±1.27	3.54±0.85	0.69±0.19	0.57±0.25	0.13±0.02
Panc.	35.85±13.46	183.12±128.38	54.75±17.63	49.98±5.86	132.9±28.99

Conclusion: RSG reduced hyperglycaemia and preserved plasma and β-cell insulin in the C57Bl/KsJ *db/db* mouse, whereas neither metformin nor glibenclamide influenced insulin parameters. RSG may therefore have the potential to exert a durable β-cell protective action in type 2 diabetes.

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NEOFORMATION OF ENDOCRINE FROM DUCTAL CELLS IN HUMAN AUTOIMMUNE DIABETES.

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Aims. In this study we evaluated the existence of proliferation and differentiation of endocrine cells from ductal cells in pancreata obtained from two young type 1 diabetic patients. **Subjects and methods.** Case 1 was a 5-year-old white female who died because of ketoacidotic coma 5 days after diagnosis. Case 2 was a 14-year-old white type 1 diabetic female in whom insulin-dependent diabetes mellitus was diagnosed 8 months before a car accident that caused her death. Pancreata from normal subjects were included as controls. Pancreatic sections were immunostained for cytokeratin-19 (Ck-19), a specific marker for human pancreatic ductal cells, and for insulin or glucagon using an indirect immunofluorescence technique and studied by laser confocal microscopy. In addition, in order to detect the possible presence of proliferative phenomena at islet or at ductal level, immunofluorescence detection of proliferating cellular nuclear antigen (PCNA) was performed employing a mouse anti-PCNA monoclonal antibody. **Results.** Immunostaining for glucagon revealed the presence of positive cells in pancreatic ducts from both Case 1 and Case 2 but not from controls. At confocal microscopy these cells revealed a double positivity when analyzed for both glucagon and Ck19, indicating that these glucagon positive cells originated from the ductal epithelium. Insulin positive cells were not detected at ductal level either in diabetic or normal pancreatic section. In pancreatic ducts from diabetic patients, but not from controls, immunostaining for PCNA showed the presence of a number of positive cells; co-staining for glucagon was observed in some of these PCNA-positive cells. **Conclusions.** This study showed differentiation and proliferation phenomena of endocrine precursor cells in ductal epithelium both at onset and at 8 months after diagnosis, suggesting that such a process takes place both in the early and late stages of the disease but it is not successful in restoring beta-cell mass. The elucidation of the mechanisms involved in the regulation of beta-cells regeneration may be of importance in developing preventive or therapeutic strategies aimed to restoring beta cell mass in the early stages of autoimmune attack.

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C-MET EXPRESSION DURING BETA CELL NEOGENESIS IN AUTOIMMUNE DIABETES.

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Aims. In this study we evaluated the expression of the hepatocyte growth factor (HGF) receptor *c-met* during beta cell neogenesis in two experimental models of autoimmune diabetes. **Materials and methods:** Non obese diabetic (NOD) female mice were killed at the following ages: 4-8 weeks, 20-25 weeks, 39-41 weeks and at diagnosis of diabetes (3 animals per group). In ten C57Bl6/J male mice, diabetes was induced by i.p. injection of low-dose streptozotocin (ld-STZ, 40 mg/Kg b/w for 5 consecutive days). Five animals were killed at day 12 and at day 24 from the beginning of the STZ treatment. In order to detect ductal *c-met* positive cells and their differentiation potential in insulin producing cells, double immunofluorescent labeling for c-*met* and CK20 or insulin was performed on 5μm thick cryostat sections. **Results.** In NOD mice, the amount and the intensity of *c-met* positive ducts was higher in pancreata from diabetic and 39-41 week-old mice in comparison with other groups. In all animals but not in 4-8 week old mice, insulin positive ductal cells were detectable. In addition, in these animals most c-*met* immunoreactive ductal cells were indeed double stained for insulin. Furthermore, pancreatic sections obtained from 39-41 week-old mice showed some small cell clusters double positive for *c-met* and insulin scattered in the exocrine parenchyma. In diabetic ld-STZ mice the amount and the intensity of *c-met* positive ducts was greater at day 24 than at day 12. Insulin positive ductal cells were detectable and most *c-met* immunoreactive ductal cells were indeed double stained with insulin. In normal mice, only a faint *c-met* staining in a small number of ductal but not insulin positive cells were observed. **Conclusions.** These results suggest a role of *c-met* receptors in ductal cell differentiation in insulin expressing cells in autoimmune diabetes. It would therefore be interesting to further explore the potentials of the HGF-*c-met* system in promoting beta cell neogenesis. The capacity to expand or mantain β cell mass using factors involved in this process could in fact represent a potential therapeutic approach for treatment of type 1 diabetes.

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THE TYROSINE KINASE BSK/IYK EXPRESSED IN ISLET CELLS CAUSES DIFFERENTIATION IN RAT PHEOCHROMOCYTOMA PC12 CELLS.

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Bsk/Iyk, a Src-family tyrosine kinase expressed in islet-cells was cloned from the mouse βTC-1 cell line. Two tyrosine residues in the C-terminal tail (Y497 and Y504) regulate Bsk activity and subcellular localisation. We have previously shown that RINm5F cells expressing constitutively active Bsk/Iyk (Y497/504F-mutated and Y504F-mutated Bsk/Iyk) display a decreased growth rate compared to control cells. Aim: To investigate if Bsk/Iyk expression affects hormone content in insulin producing RINm5F cells or neurite outgrowth in PC12 cells. Materials and Methods: RINm5F cells expressing wild-type and mutated Bsk/Iyk were subjected to Northern blot analysis for insulin and glucagon. PC12 cells expressing wild-type and mutated Bsk/Iyk were studied for their ability to differentiate (extend neurites). Results: RINm5F cells expressing Y497/504F-mutated Bsk/Iyk contained slightly less insulin mRNA compared to control cells and one RINm5F clone expressing large amounts of Y504F-mutated Bsk demonstrated elevated levels of glucagon mRNA. PC12 cells expressing wild type Bsk/Iyk show a significantly larger fraction of cells with neurites (20%) compared to control cells (1-2%) ($P<0.001$, ANOVA). Addition of 20 ng/ml NGF for 24, 48 and 72 hours increased neurite outgrowth in both control cells and Bsk/Iyk expressing cells. Transfection of PC12 cells with Y497/504F-mutated Bsk/Iyk resulted in clones of cells with long, branched neurites and flattened cell bodies. These cells ceased to grow and eventually died. Conclusions: Bsk/Iyk causes differentiation of neuronal PC12 cells. Bsk/Iyk also seems to play a role for regulating islet hormone production in RINm5F cells.

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CHRONIC TREATMENT WITH GLUCAGON LIKE-PEPTIDE-1 (GLP-1) INCREASES B-CELL MASS IN MILDLY DIABETIC RATS.

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Aims: Glucagon like-peptide-1 (GLP-1) improves glucose tolerance and partially restores glucose-induced insulin secretion both in humans with non-insulin-dependent diabetes (NIDDM) and in animal models of diabetes. We investigated whether the insulinotropic effect of GLP-1 may be related to an increase in the B-cell mass. **Materials and Methods:** we have infused non diabetic and diabetic rats with GLP-1 (1.5 pmol/kg/min) during 7 days using miniosmotic pumps. Controls were infused with saline under the same conditions. NIDDM was induced in adult rats (STZ rats) by a single i.v. injection of a low dose (35 mg/kg) of streptozotocin. **Results:** STZ rats showed a 65% decrease of the B-cell mass. In STZ GLP-1 infused rats, the B-cell mass, as measured by immunocytochemistry and stereological morphometry, was dramatically increased (4.2 ± 0.3, vs 2.0 ± 0.02 mg/pancreas; $p<0.05$). The B-cell mass was slightly but not significantly increased in non diabetic rats treated with GLP-1 (7.2 ± 0.3, vs 5.5 ± 0.5 mg/pancreas). In both groups, the proliferation of preexisting B-cells, as measured by BrdU incorporation, was unlikely responsible for this increase. On the contrary, the presence of endocrine cells within or budding from the pancreatic ductal epithelium suggests the activation of neogenesis of new endocrine cells. **Conclusion:** these data show that the GLP-1-induced increase in the insulin response to glucose may result, at least in part, from a trophic effect of GLP-1 on pancreatic islets via a stimulation of neogenic processes.

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PANCREATIC POLYPEPTIDE GENE EXPRESSION IN AR42J CELLS IS DEPENDENT ON THE TRANSCRIPTION FACTOR Nkx2.2.

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The clonal rat pancreatic acinar cell line AR42J has been reported to differentiate towards an endocrine phenotype, producing insulin and pancreatic polypeptide (PP), by treatment with growth factors, such as activin A and HGF. In our hands, growth factor treatment of the clone AR42J-B13 resulted in growth inhibition and morphological differentiation, but no insulin and PP gene transcripts could be detected. Aims: To find out which transcription factors are required for islet hormone expression in AR42J cells. Materials and Methods: mRNA expression in AR42J cells treated with activin A and HGF was investigated by Northern analysis. Transcription factor cDNAs were cloned under CMV promoter control and transfected separately and in combination. Hormone mRNA expression of the transfected cells were tested by RT-PCR. Results: mRNAs of the transcription factors Nkx6.1 and Isl1 were not found in growth factor-treated or untreated cells. Furthermore, the expression of pdx1 and Nkx2.2 was very low in comparison with INS-1 and RINm5F rat insulinoma cell lines. cDNAs encoding the above mentioned transcription factors were transfected with and without activin A + HGF treatment. The synthesis of mRNAs for insulin, glucagon and somatostatin were not detectable after any transfections. However, reproducible expression of the PP gene was induced after transfection of Nkx2.2 cDNA. This was not dependent on the growth factor treatment. Conclusion: Overexpression of the transcription factor Nkx2.2, but not Nkx6.1, pdx1 or Isl1, resulted in the induction of PP expression. None of the transcription factors could induce insulin gene expression.

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IN VITRO EXPANSION OF NEONATAL PORCINE ISLET CELLS IS ASSOCIATED WITH ELEVATED EXPRESSION OF IDX-1 AND NKX 2.2.

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Aims: Neonatal porcine islets (NPI) may become a potential source of insulin-producing tissue for transplantation. NPI which consist primarily of 55% pancreatic epithelial cells and 35% endocrine cells have the inherent capacity to proliferate and differentiate, thus making them an attractive model to study islet cell neogenesis. We have recently developed a method of enhancing maturation of NPI by microencapsulation and further culture in autologous serum. This procedure results in a more rapid correction of diabetes in NPI-transplanted SCID mice. To examine the mechanism(s) responsible for this maturation process, we determined whether microencapsulation and further culture of NPI increases the proportion of islet endocrine cells and their respective hormone contents.

Materials and Methods: Nonmature NPI were microencapsulated with purified alginate, then cultured for 8 days in the presence of 10% autologous serum.

Results: Initially, nonmature NPI consist of 29.0±2.9% and 17.8±2.6% insulin and glucagon-positive cells, respectively. After encapsulation and further culture, the proportion of insulin- and glucagon-positive cells increased significantly ($p<0.05$) to 42.8±2.4% and 25.8±1.4%, respectively. When hormone content was examined, there was a 5.6±0.4-fold increase in insulin and 1.5±0.2-fold increase in glucagon. We then analyzed the encapsulated matured NPI for their expression of the homeodomain transcription factors IDX-1 and NKX 2.2 by immunocytochemistry and Western blot. We found that IDX-1 and NKX 2.2 expression in encapsulated mature NPI was higher than nonmature NPI.

Conclusion: These data demonstrate that microencapsulation and further in vitro culture of NPI results in an increased proportion of β cells and that an increased expression of IDX-1 and NKX 2.2 may contribute to this proliferative response.

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IAPP PROMOTES CELL PROLIFERATION IN THE FETAL ENDOCRINE PANCREAS

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IAPP is predominantly expressed together with insulin in pancreatic beta cells. The peptide is expressed early during embryogenesis, i. e. IAPP coexists with insulin on the 12th gestational day in the rodent fetal pancreas. Aims: In the present study, we aimed to elucidate IAPP's role in the proliferation of fetal pancreatic islet cells. Material and Methods: For this purpose, fetal islets were isolated from pregnant Sprague-Dawley rats on gestational day 21, using a collagenase digestion method. Islets were cultured in RPMI 1640 + 10% fetal calf serum (11.1 mM glucose) for 48 h, followed by a 24 h culture period in RPMI 1640 + 1% fetal calf serum (5.6 mM glucose). The islets were then exposed to rIAPP (1-1000 nM) for 24 h. IAPP's effect on DNA synthesis (^{3}H-thymidine incorporation), islet insulin content, islet insulin secretion, islet (pro)insulin synthesis, islet total protein synthesis and glucose oxidation was studied. Results: IAPP (1-1000 nM) significantly increased DNA synthesis in fetal islets (control: 3633.9 ± 661.8 dpm/µg DNA x 6 h; 1000 nM IAPP: 5065.2 ± 624.0 dpm/µg DNA x 6 h; 100 nM IAPP: 4670.3 ± 758.5 dpm/µg DNA x 6 h; 10 nM: IAPP 5157.0 ± 768.9 dpm/µg DNA x 6 h vs 1 nM IAPP: 6346.5 ± 1535.2 dpm/µg DNA x 6 h, n=11, $P<0.05$, Student's paired *t*-test). This effect was similar to that seen when 10% fetal calf serum was added. 1 nM of IAPP increased islet insulin content (192.9 ± 33.1% of control, n=6, $P<0.05$). However, no effect could be seen by IAPP on islet (pro)insulin synthesis or total protein synthesis. After 24 h exposure to IAPP, there was a trend towards an increase in medium insulin concentration (1 nM IAPP: 140.1 ± 19.5 % of control, n=5). Islet glucose oxidation rate, stimulated by glucose, was not affected by IAPP (control: 9.1 ± 0.9 vs 10 nM IAPP: 10.5 ± 0.7, n=4). Conclusion: Our findings indicate a role for IAPP as a potential regulator of proliferation in fetal pancreatic islets. The data suggest that the proliferative effect by IAPP could partly be attributed to proliferation of beta cells.

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EFFECT OF AMINO ACIDS ON ß CELLS AND ENDOTHELIAL CELLS PROLIFERATION AND IN VITRO INTERACTION BETWEEN THE TWO CELL TYPES

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When a mother is fed a low protein (LP) diet 8% instead of 20% (C), fetal β-cell proliferation capacity (PC) is reduced. Islets vascular density is also diminished. Fetal and maternal amino acids (aa) profile is disturbed and taurine (an indispensable aa for fetal development) is dramatically reduced in fetal serum. **Aims:** This study was performed to investigate the effect of aa on endothelial cells (EC) and fetal β-cells PC *in vitro*. Furthermore, we examined a possible EC/β-cell interaction. **Materials and Methods: 1)** ECV 304 (human umbilical endothelial cell line) and CR3 (rat cerebral endothelial cell line) were incubated and challenged by different concentrations of essential aa, non essential aa or taurine during 4 days. Cells were then collected for DNA analysis by fluometric assay. **2)** Fetal LP or C islets were cultured during 6 days in RPMI and challenged by a mixture of essential aa, non essential aa or taurine during 2 days. Bromodeoxyuridine was added for the last 24 hours of the culture. Islets were labelled with anti-BrDU and Ethidium Bromide; their PC measured with confocal laser scanning microscopy. **3)** A model of coculture was worked out allowing a permanent exchange through a microporous membrane. After 2 days of coculture with EC or fibroblasts, LP or C islets PC was measured as described previously. **Results: 1)** aa supplementations failed in increasing the PC of EC, while taurine alone already at physiological concentrations (200-400 µM) had a marginal effect. **2)** with 4 times the basal concentration of essential aa in the medium, LP fetal β-cells PC increased to reach normal growth levels (11.2±1.4% vs 4.8±0.5%). 2.5 mM taurine supplementation stimulates LP β-cells PC (8.7±0.6% vs 4.8±0.5%) as well as C β-cells PC (11.1±0.4% vs 8.0±0.5%). **3)** In coculture with EC, LP and C β-cells PC is increased (12.5±0.6 % vs 6.5±0.4% and 14.7±0.8% vs 8.5±0.6% resp.). However, this mitogenic effect on LP and C β-cells was also found with pancreatic fibroblasts in coculture. **Conclusions:** *In vitro* PC of fetal β-cells can be stimulated specifically by some aa as well as by surrounding cells present in the islet *in vivo* such as EC and fibroblasts. Even after a LP diet during development, lower PC of ß cells can be restored.

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MODULATION OF PLASMINOGEN ACTIVATOR ACTIVITY IN DEFECTIVE MIGRATION OF ISLET CELLS IN MICE LACKING EGF-RECEPTORS

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Aims: During pancreatic morphogenesis the endocrine cells migrate away from the ductal epithelium through the extracellular matrix (ECM) to form mature islets. This process is disturbed in mice lacking EGF receptors (EGF-R-/-). In these mice the islet cells are mainly located in streak-like structures alongside pancreatic ducts. Proper islet morphogenesis requires appropriate balance of ECM-degrading proteinases. We have analyzed the expression of certain matrix metalloproteinases (gelatinases A and B), urokinase (uPa), tissue plasminogen activator (tPa) and plasminogen activator inhibitor type 1 (PAI-1) in EGF-R-/- pancreata and in wild-type (WT) littermates. **Materials and Methods:** Equal amounts of protein from conditioned medium of embryonic day 17.5 (E17.5) and postnatal (P1) pancreata were analyzed for gelatinolytic activity by zymography. Activities of uPa, tPa and PAI-1 were detected by caseinolysis-in-agarose and reverse zymography assays. **Results:** Gelatinolytic activity of 92 kD MMP-9/gelatinase B, and 72 kD inactive form of MMP-2/gelatinase A, were detected both in E17.5 and P1 pancreata. The activated (66 kD) form of MMP-2 was detectable only at E17.5. In EGF-R-/- pancreata there was a slight (26 %) reduction of MMP-9 as compared to WT. In the amounts of inactive 72 kDa MMP-2 no significant differences were detected. However, the amount of active (66kDa) MMP-2 was about 40% smaller in EGF-R-/- pancreata than in the WT. The amounts of both uPa and tPa were markedly higher in E17.5 as compared to P1 pancreas. There were no apparent differences in the amounts of active uPa and tPa between EGF-R-/- pancreata and WT. Unexpectedly, the amount of PAI-1 was markedly higher in EGF-R-/- pancreata both at E17.5 (2.4-fold increase) and P1 (2.7-fold). **Conclusions:** PAI-1 binds and inhibits the activity of receptor-bound uPa and shortens its half-life. It may thus modulate uPa-dependent cell adhesion and migration. Migration defect seen in EGF-R-/- pancreatic islets may be a consequence of enhanced amount of PAI-1 in the pericellular area.

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REGULAR AND INSULIN-MODIFIED IVGTT PROVIDES SIMILAR C-PEPTIDE MINIMAL MODEL INDICES OF BETA CELL FUNCTION

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Aims: The C-peptide minimal model, originally proposed to assess beta-cell function during a standard IVGTT (SIVGTT), has been then also applied in studies employing insulin modified IVGTT (IMIVGTT). In order to investigate whether the use of different protocols may affect the assessment of insulin secretion, we compared the quantitative assessment of beta cell function provided by the model from SIVGTT and IMIVGTT performed in the same individuals. **Subjects and Data Analysis:** 14 normal subjects with various degree of glucose tolerance were studied twice, with SIVGTT and IMIVGTT. The minimal model was identified from plasma C-peptide and glucose concentration data to reconstruct,in each individual and for each protocol, the pattern of pancreatic insulin secretion and to estimate three sensitivity indices: Φ_1 (10^9), Φ_2($10^9 min^{-1}$) and Φ_b ($10^9 min^{-1}$)which quantify the control exerted by glucose, respectively, on 1st and 2nd phase insulin secretion and in the basal state. **Results:** Insulin secretion profiles showed similar patterns with the two tests, with a faster decline to basal in IMIVGTT. Indices were : Φ_1=130±65 (Mean±SD), Φ_2=6.9±2.3, Φ_b=4.4±1.4 in SIVGTT and Φ_1=135±77, Φ_2=6.6±2.5, Φ_b=4.0±1.4 in IMIVGTT. No significant difference in the mean of any of the indices and no bias as revealed by plotting the difference vs. mean of the individual values were present. These results indicate that the differences observed in the secretion profiles between the two tests are only due to the differences in the glucose concentration profiles, since the sensitivities to glucose of pancreatic secretion are virtually the same. **Conclusions:** Both SIVGTT and IMIVGTT provide the same C-peptide minimal model assessment of beta cell function. Since IMIVGTT is increasingly used to measure insulin sensitivity in clinical studies with the minimal model of glucose kinetics, these results show that a detailed quantitative assessment of beta cell function can be simultaneously obtained also for this test by using the C-peptide minimal model.

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EVALUATION OF THE "GLP-1 TEST" IN PATIENTS WITH TYPE 2 DIABETES AND HEALTHY SUBJECTS.

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Aim/Hypothesis. β-cell secretory capacity is often evaluated with the glucagon test or a meal test. However, glucagon-like peptide-1(GLP-1) is the most insulinotropic hormone known, and the effect is preserved in patients with type II diabetes. Methods 1. We compared i.v. bolus injections of 2.5, 5, 15 and 25 nmol of GLP-1 with glucagon (1 mg i.v.) and a standard meal (2370kJ) in 6 type II diabetic patients and 6 matched controls. Results 1. Peak insulin and C-peptide concentrations for the patients were similar after the meal, 2.5 nmol of GLP-1 and glucagon. Methods 2. A further 6 patients and 6 controls were then included and, in addition, we performed a combined glucose+GLP-1 infusion, where plasma glucose was increased to 15 mmol/l before injection of 2.5 nmol of GLP-1. Results 2. Peak insulin (and C-peptide) concentrations (patients): meal 277±42(2181±261); GLP-1 390±74(2144±254); glucagon 329±50(1780±160); glucose+GLP-1 465±87(2384±299)pmol/l; (controls): 543±89(2873±210); 356±51(2001±130); 420±61(1995±99) and 1412±187(4391±416)pmol/l. Side effects (nausea) were less with GLP-1 than with glucagon. Methods 3. 8 patients and 8 controls, responses to glucose+GLP-1 and to a 30 mmol/l hyperglycaemic clamp + 5g arginine were compared. Results 3. Peak insulin (and C-peptide) concentrations (patients): glucose+GLP-1 475±141(2295±379); arginine 816±268(3043±508)pmol/l; (controls): 1403±308(4053±533) and 2384±452(6047±652)pmol/l. Conclusions: GLP-1 (2.5 nmol=9μg) elicits similar secretory responses to 1 mg glucagon (but fewer side effects) and a standard meal. Acute elevation of plasma glucose to 15 mmol/l does not enhance the response further in the patients. The incremental response is similar to that elicited by arginine, but hyperglycaemic clamp had an additional effect.

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INDOMETHACIN DECREASES INSULIN SECRETION IN TYPE 2 DIABETES MELLITUS

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Aims: In healthy subjects, basal endogenous glucose production is partly regulated by paracrine intrahepatic factors. Administration of Indomethacin, a prostaglandin synthesis inhibitor, resulted in a transient stimulation of endogenous glucose production without changes in glucoregulatory hormone concentrations. It is unknown whether similar paracrine factors influence basal endogenous glucose production secretion in Type 2 Diabetes Mellitus. **Materials and Methods:** The effects of 150 mg indomethacin, a non-endocrine stimulator of glucose production in healthy adults, on endogenous glucose production were measured in a randomized placebo controlled study in patients with Type 2 Diabetes Mellitus (3 men and 3 women, mean age 58.5 yrs and mean BMI 28.6 kg/m^2). Glucose production was measured before and during 6 hours after administration of indomethacin/placebo, by primed, continuous infusion of [6,6-$^2H_2$]glucose. **Results:** after indomethacin, plasma glucose concentration and glucose production increased in all subjects by 14 % (from 11.2 ± 1.7 to a maximum of 12.8 ± 1.7 mmol/L) ($p<0.05$)) and 48 % (from 12.0 ± 1.7 to a maximum of 17.8 ± 1.9 μmol/kg/min) ($p<0.05$), resp. In the control experiment, plasma glucose concentration and endogenous glucose production declined gradually in all subjects by 22 % (from 10.3 ± 1.6 mmol/L to 8.0 ± 0.9 mmol/L) ($p<0.05$) and 17 % (from 10.8 ± 1.4 to 9.0 ± 0.6 μmol/kg/min) ($p<0.05$), resp. The stimulation of glucose production coincided with inhibition of insulin secretion by 52 % within one hour after administration of indomethacin (from 78 ± 11 pmol/L to a nadir of 38 ± pmol/L) ($p<0.05$). In the control experiment insulin secretion decreased gradually by 18 % (from 88 ± 15 pmol/l to 72 ± 17 pmol/L) ($p<0.05$). **Conclusions:** indomethacin inhibits insulin secretion and stimulates endogenous glucose production through mechanisms that seem to be related to inhibition of prostaglandin synthesis. These results also indicate that drugs like indomethacin may impair glucoregulation in Type 2 diabetes mellitus.

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ACUTE REDUCTION OF FATTY ACIDS INCREASES INSULIN SECRETION IN TYPE-2 DIABETES.

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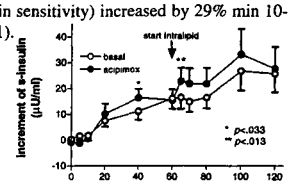
Aims: Glucose-stimulated insulin secretion (GSIS) is enhanced by an acute increase of non-esterified-fatty-acids (NEFA), however *in vitro* studies have demonstrated that prolonged elevation of NEFA has a negative effect on GSIS which is coupled to increased fatty acid oxidation. To test for NEFA effects in type-2 diabetes where basal NEFA values are increased, we measured GSIS in type-2 diabetics with hypertriglyceridemia (>2.2mmol/l) with or without reducing NEFA levels with the nicotinic acid derivative Acipimox.

Materials and Methods: Type-2 diabetic patients (10 males, 11 females; age 55,9 ± 2,4years, body weight 93,0 ± 3,3kg, f-bglc 10,6 ± 0,5mM, HbA1c 7,9 ± 0,3%,C-peptide 1,19± 0,10nmol/l, triglycerides 4.6 ± 0,7mmol/l) underwent a hyperglycemic clamp (11mM, 120 minutes). During min 60-120 Intralipid (20%,1mL/min) and Heparin (0.4U/kg/min) were co-infused with glucose. Sixty minutes before clamps patients ingested either placebo (basal) or 250mg acipimox. Tests were performed in randomized orders with an interval of 2-4 weeks.

Results: Following Acipimox NEFA levels were decreased at min 0 by 48%, whereas infused glucose (a measure of insulin sensitivity) increased by 29% min 10-59 ($p<.003$) and by 12% min 60-120 ($p<.021$). Acipimox enhanced insulin secretion as indicated in fig. and as insulin area min 60-120 by 250,7 ± 154,2 ($p<.023$).



Conclusions These results are consistent with long term elevated NEFA exerting a tonic negative influence on insulin secretion in type-2 diabetes patients.

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INSULIN SECRETION AND INSULIN RESISTANCE AFTER RENAL TRANSPLANTATION

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Aims: The prevalence of post-renal transplantation diabetes mellitus(PTDM) is known to be much higher than that of normal populations. The exact mechanism whether insulin resistance or insulin deficiency is predominant factor in PTDM is still unknown. The aim of our study is to investigate the pathogenesis and possible risk factors for PTDM in Korea. **Materials and Methods:** 75 g OGTT and plasma glucose disappearance rate(kitt: %/min) after iv injection of regular insulin(0.1U/kg) were performed in 81 patients participated 1 week before, and between 3 months and 12 months after transplantation. We checked the insulin(I) and the proinsulin. The changes of the fat and the muscle were determined by Bioimpedence method. **Results:** Impared glucose tolerance (IGT) after transplantation was developed in 44% and PTDM in 24%. Before transplantation, the basal and 2 hr post-load glucose level of IGT and PTDM patients was significantly higher than those of normal glucose tolerance(NGT) patients($p<0.05$). The proinsulin/insulin ratio and insulin levels of 30 min after OGTT in IGT and PTDM group were significantly lower($p<0.05$), and $\Delta I/\Delta G$(30min) were significantly lower than NGT group($p<0.05$). After transplantation, the fat contents(%) of IGT and PTDM group were significantly higher and the muscle contents were significantly decreased in IGT and PTDM group after transplantation($p<0.05$). The index of insulin sensitivity, kitt, was increased in all the groups, but more pronunced in NGT group after transplantation($p<0.05$). The sum of the insulin responding to OGTT was significantly lower in PTDM than in NGT($p<0.01$). **Conclusions:** These results suggest that the glucose, proinsulin and insulin response to OGTT before transplantation may be the predictors of whether PTDM develop or not, and the defect of insulin secretion may be more important factors in PTDM than insulin resistance.

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PROINSULIN SECRETION INCREASES AS BLOOD GLUCOSE CONTROL DETERIORATES BUT IS NOT INFLUENCED BY OBESITY

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Aims : We evaluated the relationship between proinsulin (PI) and body fat distribution or blood glucose control of patients with type 2 diabetes. **Methods :** The present study included 70 patients with type 2 diabetes not receiving insulin injection, 42 men and 28 women aged 56.1 on the average. The following parameters were determined in each patient : fasting blood glucose (FBG), HbAlc, fasting immunoreactive insulin (IRI : EIA), fasting PI (PI : RIA), molar PI/I ratio (PI/I), body mass index (BMI), body fat ratio (BFR), visceral fat area (V), subcutaneous fat area (S), and V/S ratio (V/S). BFR was culculated by dividing the fat weight by body weight. V and S values were calculated from CT images of the cross section of the umbilicus. Spearman's correlation coefficient and Scheffe's test (ANOVA) were used for statistical analyses. **Results :** IRI was significantly correlated with BMI ($r=0.46$, $p<0.001$), BFR ($r=0.47$, $p<0.001$) and S ($r=0.32$, $p<0.01$) but not with FBG or HbAlc. PI was significantly correlated with BMI ($r=0.24$, $p=0.02$), BFR ($r=0.42$, $p<0.001$), and V ($r=0.25$, $p=0.04$), as well as FBG ($r=0.30$, $p<0.01$) and HbAlc ($r=0.30$, $p<0.01$). PI/I was significantly correlated with FBG ($r=0.37$, $p<0.001$) and HbAlc ($r=0.49$, $p<0.001$) but not with BMI, BFR, V, S or V/S. PI increased significantly in patients with FBG $\geqq$ 9.0 (mmol/L) compared to those with FBG<7.0. PI/I was comparable in patients with FBG<7.0 and those with 7.0 $\leqq$ FBG<9.0 but increased significantly in patients with FBG $\geqq$ 9.0 compared to the other two groups. However, FBG showed no effect on IRI. PI/I increased with increases in HbAlc, and increases were particularly marked in patients with HbAlc $\geqq$ 10%. **Conclusion :** PI secretion increased with increases in BMI, body fat ratio, and V. However, this increase was due to increased secretion of IRI, and PI secretion relative to IRI secretion (PI/I) was not related with obesity or the body fat distribution. On the other hand, PI and PI/I levels increased with increases in FBG and HbAlc levels. The PI/I ratio increased markedly in patients with FBG $\geqq$ 9.0 mmol/L and those with HbAlc $\geqq$ 10%. These findings suggest that poor blood glucose control loads the function of β cells and that obesity and the body fat distribution are not related to PI secretion.

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EX VIVO AND IN VITRO EFFECTS OF INSULIN AND C-PEPTIDE ON Na/K ATPase ACTIVITY IN RED BLOOD CELL MEMBRANES OF TYPE 1 DIABETIC PATIENTS.

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A decrease in Na/K ATPase activity is observed in several tissues of type1 diabetic patients. It is supposed to play a role in the development of some long term complications. Insulin infusions may restore this enzyme activity in red blood cell (RBC) , and arguments have been developped for a similar role of C-peptide. **Aims:** the aim of this study was to determine whether insulin acts directly on the RBC's enzyme, and to evaluate the effect of C-peptide. **Materiels and Methods:** 35 C-peptide negative type 1 diabetic patients were studied (blood glucose 207±20mg/dl, HbA1c 8.9±0.4%,means±SEM). Blood samples were obtained in the morning before breakfast and injection of insulin, RBC were resuspended in their own plasma and incubated in presence or absence of insulin (50uU/ml) or C-peptide (6nM). The enzymatic activity was assessed by 2 techniques: 1) On intact red cells, heat production by the enzyme-induced hydrolysis of ATP was measured inside a thermostated microcalorimeter at 37°C (ex vivo study). 2) After incubation at 37°C, RBC membranes were isolated and Na/K ATPase activity assessed by measurement of inorganic phosphate released at saturating concentrations of all substrates (in vitro study). **Results:** Ex vivo, Na/K ATPase activity which was lower in type 1 diabetic patients than in control subjects (12±0.05 vs 16±0.07 mW/l RBC) was significantly increased by insulin (12±0.5 vs 15±0.9, $p=0.013$, $n=23$) but not by C-peptide (12±0.7 vs 13±0.9 $n=12$). In vitro, insulin and C-peptide increased significantly Na/K ATPase activity (338±27 and 356±35 respectively vs 262±24 nM Pi.mg protein^{-1}.h^{-1}, $p=0.01$, $n=12$). **Conclusions:** these results demonstrate a direct effect of insulin on RBC's Na/K ATPase with a restoration of this activity in type 1 diabetic patients. The stimulating effect of C-peptide observed in vitro on RBC's Na/K ATPase supports the hypothesis for a physiological role of C-peptide.

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MAGNESIUM SEQUESTRATION IN RED BLOOD CELLS AS A MECHANISM OF HYPOMAGNESEMIA IN DIABETIC PATIENTS

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Aims: Hypomagnesemia is implicated in the etiopathogenesis of diabetes mellitus and its complications, both as a cause and consequence. However, the mechanism of hypomagnesemia in diabetes is not yet clearly understood. We have tested the hypothesis that red cell sequestration, induced by hyperglycemia, is a major cause of hypomagnesemia in diabetes and it is alleviated by insulin therapy. **Subjects and methods:** Twenty-three untreated (age in yrs: 25±4; BMI: 20.23±5.15, M±SD) and 32 insulin-treated (age in yrs: 22±4 and BMI: 19.6±3.12) diabetic subjects were studied for their blood glucose, serum Mg and red cell Mg values. RBCs were washed thrice with 300 mM ice-cold sucrose solution to get rid of extracellular magnesium and then homogenized using 1% tritonX100. The Mg content in the homogenized cells was measured by an enzymetic-colorimetric method. Protein content of the cells was measured by a detergent compatible kit using spectophotometric technique. Glucose was measured by Glucose-oxidase method. **Results:** The Untreated diabetic patients had considerably higher degree of fasting hyperglycemia compared to the Treated group (mmol/l, M±SD, 14.93±6.87 in Untreated vs 9.63±4.27), however the two groups showed almost similar postprandial glycemic values after 2 hrs of oral glucose load (mmol/l, M±SD, 24.08±5.97 vs 22.84±8.06). Serum magnesium (mmol/l, M±SD) was higher in the Untreated group (0.96±0.12) compared to the Treated group (0.92±0.10) but it did not differ statistically. RBC magnesium (µmol/mg of protein, M±SD) in the Untreated group (0.11±4.97) was significantly higher compared to the Treated group (0.05±0.001). Serum to RBC Mg ratio (mmol/ µmol Mg per mg RBC total protein) in the Treated group (12.80±2.75) was significantly higher compared to the Untreated group (9.875±4.92, t= -2.80, p=0.007). **Conclusions:** a) Hyperglycemia causes sequestration of Mg in the red blood cells and b) treatment with insulin seems to release some Mg from red blood cells and thus improve the homeostasis in the patients.

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Cytokine Induced β-Cell Destruction

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GLUTATHIONE DEPLETION INHIBITS IL-1β-INDUCED NO PRODUCTION BY REDUCING iNOS GENE EXPRESSION

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Interleukin-1β (IL-1β) may be an important mediator of pancreatic β cell destruction in insulin-dependent diabetes mellitus. Cytokine-induced formation of free oxygen and nitric oxide radicals has been implicated in IL-1β-induced cytotoxicity. Glutathione (GSH) participates in the antioxidant defense system. In this study we investigated the influence of GSH depletion on IL-1β-induced NO production in rat islets, purified rat β cells and RINm5F cells. In purified rat β cells preincubation with L-buthionine-S,R-sulfoximine (BSO), an inhibitor of the key enzyme in GSH synthesis, dose-dependently decreased cytokine-stimulated nitrite release with a maximal effect at 1 mM of BSO (36 %). An inhibitor of glutathione reductase 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) suppressed IL-1β-mediated nitrite production in isolated rat islets by 34 % at 0.5 mM of BCNU. The thiol depletor diethyl maleate (DEM) dose-dependently decreased cytokine-induced nitrite formation in isolated rat islets and completely blocked it at 50 μM of DEM. One mM of BSO inhibited IL-1β-induced nitrite release and iNOS gene promoter activity in RINm5F cells (26 % and 17 %, respectively) and iNOS mRNA expression in isolated rat islets (53 %). We conclude that GSH regulates IL-1β-induced NO production in rat islets, purified β cells and insulinoma cells by modulation of iNOS gene expression and that other reductants than GSH take part in this process.

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IL-18 mRNA, BUT NOT IL-18 RECEPTOR mRNA, IS CONSTITUTIVELY EXPRESSED IN ISLET BETA-CELLS.

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It has been demonstrated that interleukin-18 (IL-18) mRNA is expressed in islets of NOD mice during early stages of insulitis and that systemic treatment of NOD mice with IL-18 suppresses diabetes development. **Aims**: To investigate whether there is any effect of IL-18 or whether IL-18 modulate IL-1β effect on islet function, and whether IL-18 and its receptor (IL-18R) are expressed in isolated islet beta-cells. **Materials and Methods**: Insulin release and nitrite production from isolated islets of newborn Wistar rats were measured after incubation with or without cytokines. RT-PCR was used to quantitate mRNA expression of IL-18 and IL-18Rβ, also called accessory protein-like (AcPL). **Results**: There were no significant effects of rmIL-18 (0.625, 1.25, 2.5, 5,10 nM) alone on accumulated or glucose-challenged insulin release or nitrite production after 24 h. 15 pg/ml of rhIL-1β as well as rrIFN-γ (200 U/ml)+rhTNF-α (250 U/ml) significantly increased nitrite production and inhibited both accumulated and glucose-challenged insulin release. However, rmIL-18 failed to modulate the above effects caused by IL-1β or IFN-γ+TNF-α. Although IL-12 can induce IL-18R expression in T lymphocytes, 24-h rmIL-12 preincubation failed to prime islets for effects of 10 nM of rmIL-18 (also rrIL-18) itself or in synergy with rhIL-1β on insulin release and nitrite production. RT-PCR showed that IL-18 mRNA expression was found in RIN cells and in isolated islets. Further, the IL-18R signaling component IL-18Rβ mRNA was expressed only in rat mononuclear cells (positive control) and not in RIN cells even after exposure to IL-1β or/and IFN-γ+TNF-α. **Conclusions**: IL-18R is not expressed constitutively or after pro-inflammatory cytokine exposure in rat islet beta-cells. The physiological activity and pathological role of IL-18 originating from islet beta-cells deserves further investigation.

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THE ROLE FOR INTERFERON REGULATORY FACTOR-1 (IRF-1) IN CYTOKINE-INDUCED MURINE β-CELL mRNA EXPRESSION AND DEATH

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Aims: Combination of cytokines induce nitric oxide (NO) production and cell death in islet cells. These events are preceded by increased expression of the transcription factor IRF-1. We presently utilized an IRF-1 knockout mouse (IRF-1$^{-/-}$) to investigate the role for IRF-1 in cytokine-induced islet- and β-cell gene expression and cell death. **Materials and Methods:** Pancreatic islets or FACS-purified β-cells were isolated from wild type (wt) or IRF-1$^{-/-}$ mice. These cells were exposed for different time points to IL-1β (50 U/ml), IFN-γ (1000 U/ml) and/or TNF-α (1000 U/ml) before being harvested for determination of viability (by nuclear dyes) and mRNA expression (by RT-PCR with specific primers). **Results:** Following a 24 h exposure to IL-1β or IL-1β + IFN-γ pancreatic islets isolated from IRF-1$^{-/-}$ mice presented a 30-50 % reduction in medium nitrite accumulation and inducible NO-synthase (iNOS) expression ($p<0.01$ vs wt cell, n=7-8) and better preserved insulin mRNA expression. Interestingly, both wt and IRF-1$^{-/-}$ purified β-cells failed to produce NO in response to IL-1β alone, but presented a similar increase in nitrite accumulation and iNOS expression following exposure to IL-1β + IFN-γ. The basal expression of MHC class I mRNA was lower in IRF-1$^{-/-}$ islet cells (30% reduction; $P<0.05$ vs wt cells, n=7), but there was a similar 2-4 fold-increase in MHC expression in islet cells from both strains following cytokine exposure. Treatment of whole islets for 3 days with IL-1β + IFN-γ induced significantly more islet cell death in wt than in IRF-1$^{-/-}$ mice (respectively 85 ± 3 % vs 31 ± 4 % dead cells; $P<0.001$; n=4). On the other hand, prolonged exposure (3-9 days) of FACS-purified β-cells to the same cytokines, or combination of 3 cytokines, lead to a similar increase in cell death in both IRF-1$^{-/-}$ and wt islets. **Conclusions:** IRF-1 contributes for cytokine-induced islet iNOS expression and cell death. These effects are absent in purified β-cells, suggesting that IRF-1 may mediate its effects on whole islets via activation of non-endocrine cells (e.g. macrophages and ductal cells) present in these preparations.

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MOLECULAR CHARACTERIZATION OF β-CELL MATURATION AND CYTOKINE INDUCED β-CELL TOXICITY.

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Background: During ontogeny of the pancreas, all four endocrine phenotypes in the islets of Langerhans (α, β, δ and PP cells) develop from the same stem cells. Cytokines, in particular interleukin-1β (IL-1β), are specifically toxic to β-cells in the islets of Langerhans, in part through induction of free radicals, e.g. nitric oxide. In previous analyses of isolated rat islets exposed to cytokines, we have by 2D-gel electrophoresis and mass spectrometry (MS) identified 105 out of 2000 proteins which reproducibly changed expression levels in response to IL-1β. In recent studies using a pluripotent endocrine *in vitro* cultured cell-system, the MSL-cells, which dependent on culture conditions may present as either a glucagon (NHI-glu) or insulin (NHI-ins) expressing phenotype, we have demonstrated that the maturation into the β-cell phenotype is associated with an increased sensitivity to the toxic effects of IL-1β as well as an acquired sensitivity to the β-cell toxin streptozotocin. **Aim:** Using the two maturation stages of the MSL-cells to identify proteins/protein modifications associated with β-cell maturation, and establish their potential involvement in cytokine mediated β-cell destruction. **Results:** The MSL-cell system has been analyzed by 2D-gel electrophoresis and until now the basic part (pI 6.5-10.5) of the gels containing most of the regulatory proteins has been analyzed. When comparing the two phenotypes 53 of 652 protein-spots showed an altered expression level ($p<0.01$, n=4), of these 12 spots was completely suppressed in the NHI-ins phenotype. Cytokine exposure of the β-cell phenotype resulted in altered expression level of 26 spots, out of 641 detectable, and for the NHI-glu phenotype 31 out of 655 was altered. Of these 4 spots were altered after cytokine exposure in both phenotype, and 6 were also altered during maturation. **Conclusion:** We have identified a specific set of protein-spots altered during β-cell maturation as well as in response to cytokines. MS identification of the proteins behind these spots and further characterization may yield important information to novel strategies towards intervention, prevention and/or islet transplantation in IDDM.

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EFFECTS OF MELATONIN ON STREPTOZOTOCIN AND INTERLEUKIN-1β (IL-1β) INDUCED β-CELL DAMAGE.

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Exposure of rodent pancreatic islets to IL-1β or streptozotocin (STZ) suppresses β-cell function. Activation of inducible nitric oxide synthase (iNOS) and subsequent nitric oxide (NO) formation is suggested to mediate IL-1β induced β-cell impairment. DNA alkylation leading to extensive DNA repair and depletion of intracellular NAD may mediate STZ toxicity. The neurohormone melatonin has been suggested both to protect against development of autoimmune diabetes in NOD mice and to counteract STZ induced diabetes complications in the rat. Recently, melatonin was suggested to inhibit NO formation by affecting iNOS promotor activity in macrophages. **Aims:** To investigate if melatonin affects IL-1β or STZ induced damage on rat islets *in vitro*. **Materials and Methods:** Pancreatic islets were isolated from Sprague-Dawley rats and cultured for 6-7 days. For IL-1β experiments, islets were preincubated with melatonin (100 μM to 1 mM) for 30 min, then exposed to IL-1β (25 U/ml) for 48 h in culture. NO formation was analysed as nitrite accumulation into medium. Insulin secretion into medium was measured and islet mitocondrial function was analysed by measuring glucose oxidation rates. For STZ experiments, islets were preincubated with melatonin (10 μM to 1 mM) for 30 min and then exposed to STZ (0.5 mM) for 30 minutes. After culture in fresh medium for 18 h islet mitochondrial function was measured as above. **Results:** Melatonin at 1 mM significantly protected against STZ induced inhibition of mitochondrial function (control 319 ± 27 pmol glucose/10 islets; STZ 105 ± 39 pmol glucose/10 islets vs STZ+1mM melatonin 263 ± 60 pmol glucose/10 islets, n=5, $P<0.01$, ANOVA). However, melatonin did not protect against IL-1β induced NO formation and had no effect on impaired glucose oxidation rates or decreased insulin secreation. **Conclusion:** Melatonin does not protect islets against IL-1β induced impairment, but may protect versus STZ induced β-cell damage.

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ENDOGENOUS AND CHEMICALLY-GENERATED NITRIC OXIDE ALTERS PROTEIN EXPRESSION IN RAT ISLETS

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Interleukin-1β (IL-1β) treatment of neonatal rat islets for 24h induces changes in the expression of 105 out of 2,200 detectable proteins as determined by two-dimensional (2D) gel electrophoresis. Nitric oxide (NO) has been implicated as a mediator of IL-1β effects in insulin-containing cell lines and rat islets. **Aims:** To a) determine the involvement of NO in IL-1β-induced alterations in protein expression, and b) investigate the effects of chemically-generated NO on protein expression by 2D gel electrophoresis of neonatal rat islet samples. **Methods:** Precultured rat islets were exposed to 150pg/ml IL-1β for 24h in the absence of nitric oxide formation or to the NO donor S-nitrosoglutathione (GSNO). Islet proteins were then labelled for 4h with [^{35}S]methionine and separated by 2D gel electrophoresis. Computer-based analysis of the gels was performed using the BioImage program. **Results:** Of the 105 proteins affected by IL-1β, 4 were found to have significantly altered protein expression when nitric oxide production was inhibited ($p<0.01$). Three of these were positively identified by mass spectrometry as heat shock protein 70, protein disulphide isomerase and glyceraldehyde-3-phosphate dehydrogenase. Computer-based analysis of gels from GSNO-exposed islets revealed alterations in the expression of 20 of a total of 1,600 detectable proteins compared to untreated islets ($p<0.01$). **Conclusion:** The majority of IL-1β-induced protein expression changes are NO-independent. Nitric oxide, when applied exogenously for 24h, alters the subsequent expression of a relatively small number of proteins.

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POSSIBLE DIRECT PROTECTIVE EFFECT OF Th2 AND Th3 CYTOKINES ON Th1 CYTOKINE-INDUCED FUNCTIONAL DAMAGE OF ISOLATED HUMAN ISLETS.

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Aims: In-vivo studies in rodents have suggested that Th2 and Th3 cytokines can be effective in reducing Th1 cytokine-induced islet damage. In the present report we evaluated whether Th2 (IL-4 plus IL-10) and Th3 (TGF-1beta) cytokines may counteract the functional derangements induced by Th1 cytokines (IL-1beta plus IFN-gamma plus TNF-alpha) in isolated human islets in-vitro. Materials and Methods: Human islets were prepared by collagenase digestion and density gradient purification from four pancreases, and incubated for 12 hours either with or without IL-1beta (50 U/ml), plus IFN-gamma (1,000 U/ml), plus TNF-alpha (1,000 U/ml). At the end of incubation the islets were sequentially challenged with 3.3 and 16.7 mM glucose. Islet cell survival was assessed by MTT staining and/or the TUNEL technique. Islet expression of mRNA encoding for the Bcl-2 protein was assessed by RT-PCR. Results: In the absence of cytokines (control islets, Ctrl), insulin release (IR, μU/ml) was 87±52 (mean±SD) at 3.3 mM glucose, and 217±102 at 16.7 mM glucose ($p<0.01$ vs 3.3 mM glucose). The islets pre-incubated with Th1 cytokines showed a slight increase of IR at 3.3 mM glucose (129±109, NS vs Ctrl) and a significant decrease of IR at 16.7 mM glucose (109±111, $p<0.05$ vs Ctrl), thus confirming the functional damage induced by Th1 cytokines on isolated human islets. The addition of IL-4 (500 U/ml) plus IL-10 (100U/ml) in the medium during the incubation period caused no major change of IR at 3.3 mM glucose (132±57, NS vs Ctrl), and restored IR in response to 16.7 mM glucose (220±31, NS vs Ctrl). Similar effects were observed with the addition of TGF-1beta (5 ng/ml): in this case, IR was 132±86 (NS vs Ctrl) at 3.3 mM glucose, and 195±111 (NS vs Ctrl) at 16.7 mM glucose. No major difference in islet cell survival or Bcl-2 mRNA expression was observed under the different incubation conditions. Conclusions: These results suggest that Th2 and Th3 cytokines may have a direct protective effect on the insulin secretory function of human islets exposed to Th1 cytokines.

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ß-CELL SURFACE MOLECULE EXPRESSION IS MODULATED BY A THIAZOLIDINEDIONE

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Besides improving insulin sensitivity in type 2-diabetes, thiazolidinediones have been suggested to exert an anti-inflammatory action in pancreatic islets. – Therefore, in an invitro study, potential effects of troglitazone (TGZ) were explored on the expression of cell surface molecules on ß-cells. 500 islets prepared from 2 week-old rats (collagenase technique) were, after 4-days-preculture, exposed for 24 h to IL1-ß (20 U/ml) to upregulate MHC class I (OX18) and adhesion molecule (ICAM-1) expression. In a separate group, the islets were additionally treated with TGZ (10 μM); control islets were cultured with DMSO, the solvent of TGZ. ß-cell expression of OX18 and ICAM-1 was measured by flow cytometry following double immunostaining of the islet cells with monoclonal antibodies against ß-cells and the respective surface antigen. Also, insulin secretion was measured during and after in vitro treatments. - Results: Exposure of the islets to IL1-ß caused an increase of OX18- and of ICAM-1 positive ß-cells (controls vs. IL1-ß: 21.16 ± 1.91 vs. 62.41 ± 2.75 % for OX 18, and 5.45 ± 0.57 vs. 60.67 ± 1.56 % for ICAM-1, $p<0.01$); also, glucose-stimulated insulin secretion was reduced by 40 % ($p<0.01$). The latter effect was correlated to an elevated islet nitrite production. Neither TGZ alone nor the solvent, DMSO, did significantly alter the expression of surface molecules or the secretion of insulin. TGZ did, however, reduce the IL1-ß-induced expression of OX18 by 16% ($p<0.05$), and that of ICAM-1 by 54 % ($p<0.01$) when applied simultaneously with IL1-ß. Also, TGZ completely prevented IL1-ß induced nitrite generation by the islets cells, but did not counteract the impairment of glucose-stimulated insulin secretion. In addition, the IL1ß-induced expression of both OX18 and ICAM-1 seemed to depend upon ambient glucose concentration in that the expression was lower at a glucose level of 5.5 as compared to 11.1 mmol/l. The expression of MHC class II (OX6) was not influenced by any of the procedures described.- Conclusion: IL1ß induces functional damage of ß-cells which cannot be prevented by co-culture with TGZ as such. TGZ may reduce the IL1ß-induced expression of ß-cell surface molecules indicating potential immune-modulating properties of the drug. Finally, less active ß-cells appear to be less susceptible to damage by cytokines which may influence the potential protective effect of TGZ.

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BETA CELLS CONTRIBUTE TO THEIR OWN DEMISE: THE ROLE OF IL-18 IN TYPE 1 DIABETES.

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Pro-inflammatory cytokines (e.g. IL-1β, IFNγ and TNFα) and toxic free radicals play a major role early in the pathogenesis of type 1 diabetes. The recently characterized interleukin 18 induces IFNγ production in T-cells and, therefore, amplifies the inflammatory response. Here we demonstrate that the exposure of wild type mouse islets of Langerhans or of a β-cell line (NIT-1) to a combination of IL-1β (15U/ml), IFNγ (80U/ml) and TNFα (10U/ml) induces the transcription of IL-18 specific mRNA. To demonstrate function, a bioassay was performed to detect IL-18 mediated secretion: supernatant from islets and NIT-1 cells, respectively, exposed to pro-inflammaroty cytokines significantly induce IFNγ production by T-cells. Since interleukin-1β converting enyme (ICE) is responsible for the processing of pro-IL-18 to functionally mature IL-18 both islets and NIT-1 cells were preincubated with the specific ICE inhibitor Z-VAD-FMK. This treatment resulted in the blocking of IL-18 processing and subsequently in the lack of IFNγ induction. Analysis of islets and NIT-1 cells exposed for 24 hours to different concentrations of IL-18 did not change insulin release, nitric oxide production and Fas/FasL epression, suggesting that β-cells might not be responsive to IL-18. RT-PCR analysis for the expression of IL-18 receptor underscores this contention. We therefore hypothesise that the production of IL-18 by β-cells exposed to pro-inflammatory cytokines enhances the Th1 production of IFNγ and thus contributes to the specific destruction of β-cells.

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PANCREATIC AUTOIMMUNITY IN PATIENTS WITH PRIMARY HEMOCHROMATOSIS.

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Primary hemochromatosis and hemochromatosis secondary to transfusions, as in thalassemia major have common the damaging effects caused by iron deposition in tissues. High incidence of diabetes is also observed in primary hemochromatosis. We have previously reported that a high frequency of islet cell antibodies (ICA) is detected in individuals with thalassemia major. The oxidizing activity of iron has been hypothesized to play a role in inducing the autoimmune mechanisms that give rise to diabetes. **Aims**: the aim of this work is to see if autoimmunity directed against pancreatic islet is detectable in diseases due to a primary defect of iron metabolism. **Materials and Methods**: 15 individuals (13 males, 2 females; average age 50.9±14 years) with primary hemochromatosis, of whom nine had diabetes, were analysed by indirect immunofluorescence for the presence of ICA, anti-glutamic acid decarboxylase antibodies (GADA) and anti-insulin antibodies, and by evaluation of basal serum C-peptide by radioimmunological assay. **Results**: three subjects, all diabetic, were ICA positive (20.0%); C-peptide was undetectable in one of the three cases and in only one case there was an association between anti-insulin antibodies in a patient previously treated with insulin. All patients were negative for GADA. Extra-insular fluorescence was detected in 8 cases (53,33%) consistent with exocrine tissue damage due to iron deposition and consequent induction of autoimmune reponse against aspecific antigens of extra-insular pancreatic tissue. **Conclusions**: the finding of ICA positivity, with a higher frequency than expected, within diabetic population affected by primary hemochromatosis together with the presence of features suggestive of type 1 diabetes in one the patients highlights the possible role of autoimmunity directed against pancreatic islet in the aetiology of these forms of diabetes. This finding strengthens the hypothesis that iron may play a crucial role in triggering autoimmune manifestations in both primary and secondary hemocromatosis.

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Apoptosis of β-Cells

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INVESTIGATION OF THE SUSCEPTIBILITY OF DYNABEAD IMMUNOPURIFIED ß-CELLS TO CYTOKINES

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Aim: To investigate the susceptibility of rat ß-cells versus mixed islet cells to DNA damage following cytokine exposure. **Methods:** Adult rat islets were treated for 48h with 3 cytokines (interleukin-1ß 100pM, tumour necrosis factor-α 100pM and interferon-γ 2U/ml) then dispersed into single cells using Accutase™. Cells were incubated with a ß-cell-specific cell surface antibody, an IgG, for 30min. Dynabeads M450, coated with sheep anti-mouse IgG ($0.5\text{-}1\times10^7$ beads/ml), were added to antibody-treated cells for 20min on ice. The Dynabead-coated cells were separated from the suspension by placing the tube in a Dynal MPC magnet. The comet assay was used to detect DNA damage in the ß and non-ß cell populations. **Results:** The number of ß-cells in the Dynabead-coated population was up to six times higher than in the non-Dynabead-coated cells, as determined by measuring the insulin content and cell number of each cell population. A mixed rat islet cell population showed significant DNA damage after cytokine exposure as indicated by an increase in comet length (μm) from 23.4±0.8 to 44.3±3.5 N=4, p=0.001. The Dynabead-separated ß-cells showed significant DNA damage after cytokine treatment, from 24.4±1.1 (N=5) for control to 43.7±2.7 (N=5) p<0.001. However the non-ß-cells showed similar DNA damage, comet length from 22.8±0.6 (N=5) to 46.2±3.6 (N=5) p<0.001. **Conclusions:** Rat islet ß-cells can be positively selected from a mixed cell population using immunomagnetic beads. Both ß-cells and non-ß-cells show equal susceptibility to DNA damage induced by a combination of cytokines.

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MECHANISM OF COXSACKIE VIRUS INDUCED DAMAGE IN HUMAN BETA CELLS

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Aims: Enteroviruses may be involved in the pathogenesis of IDDM, either through direct ß-cell infection or as triggers of autoimmunity. In the present study ew investigated the patterns of infection in human ß-cells by several coxsackie viruses. **Materials and Methods:** Isolated adult human islets (56% beta cells) were infected with prototype strains of coxsackie B (CBV) 3, 4 and 5, as well as coxackie A9 (CAV-9). Insulin release was measured by perifusion. Mechanisms of cell death were studied by cell viability staining, electron microscopy and in situ DNA end-labelling (TUNEL). Involvement of nitric oxide was studied by measuring medium nitrite and iNOS mRNA expression in the infected cells. **Results:** All viruses replicated well in human ß-cells, but only CBV's caused cell death. One week after infection, the insulin response to glucose or glucose plus theophylline was most severely impaired by the CBV-5 infection. CBV-3 and -4 also caused significant functional impairment, while CAV-9 infected cells responded like uninfected controls. After 2 days of infection, 36% of CBV-5 infected cells had undergone morphological changes characteristic of pyknosis, i.e. highly distorted nuclei with condensed but intact chromatin. Both mitochondria and plasma membrane were intact in these cells. Control cells showed well preserved morphology. One week after infection the majority of cells showed characteristics of secondary necrosis. 2 days after infection DNA fragmentation was found in 5.9±1.1% of CBV-5 infected ß-cell nuclei (2.1±0.3% in controls, p<0.01). CAV-9 infection did not induce DNA-fragmentation. Medium nitrite and iNOS mRNA levels were not significantly upregulated by the CBV infection. **Conclusions**: Infection of human beta cells with coxsackie B viruses causes functional impairment and cell death characterized by nuclear pyknosis. Apoptosis appears to play a minor role during a productive CBV-infection.

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INCREASED BETA CELL APOPTOSIS IN SYNGENEICALLY TRANSPLANTED ISLETS EXPOSED TO CHRONIC HYPERGLYCAEMIA

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Aim: to determine whether apoptosis is involved in the reduction of beta cell mass observed in singeneically transplanted (Tx) islets exposed to chronic hyperglycaemia. Materials and Methods: six groups of streptozotocin diabetic C57Bl/6 mice were Tx with 100 syngeneic islets, an insufficient beta cell mass to achieve normoglycaemia. Groups 1 (n=6), 2 (n=5) and 3 (n=6) remained hyperglycaemic throughout the study. Groups 4 (n=7), 5 (n=6) and 6 (n=5) were kept normoglycaemic with insulin treatment from day 7 before Tx to day 10 after Tx, and remained normoglycaemic when insulin was removed. Grafts were harvested 3 (groups 1 and 4), 10 (groups 2 and 5) and 30 (groups 3 and 6) days after Tx. Apoptosis was determined by TUNEL technique and expressed as percentage of positive beta cells. Beta cell mass was measured by point counting morphometry. Results: beta cell apoptosis was increased on day three after Tx (group 1: 0.40 ± 0.05 %, group 4: 0.38 ± 0.05 %) compared to apoptosis in pancreas of control animals (0.08 ± 0.03%, $p<0.005$). Beta cell apoptosis remained increased 30 days after Tx in hyperglycaemic mice (group 3: 0.38 ± 0.06%) compared to normoglycaemic mice (group 6: 0.12 ± 0.03%, $p<0.005$). Beta cell necrosis was high on day three (group 1: 29.0%, group 4: 31.5%), but it was almost undetectable on day 10 and 30. Thirty days after Tx beta cell mass was reduced in hyperglycaemic mice (group 3: 0.039 ± 0.008 mg) compared to the initially Tx mass (0.121 ± 0.010 mg, $p<0.0005$). In contrast, in normoglycaemic mice it was similar to the initially Tx mass (group 6: 0.105 ± 0.010 mg). Conclusions: beta cell apoptosis was increased in the first days after syngeneic islet Tx both in hyperglycaemic and normoglycaemic groups, but beta cell necrosis was the main contributor to beta cell death. Beta cell apoptosis remained increased 30 days after Tx in hyperglycaemic animals, suggesting that apoptosis is involved in the reduction of Tx beta cell mass in Tx islets exposed to chronic hyperglycaemia.

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EFFECTS OF GROWTH HORMONE AND PROLACTIN ON APOPTOTIC DEATH OF INS-1 CELLS

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Aims: Growth hormone (GH) and its related peptide prolactin (PRL) are well-known stimulatory peptides on growth and the function of pancreatic β-cells. In this study, we investigated effects of these hormones on apoptotic death of insulin-secreting cells using a rat insulinoma cell line INS-1. **Materials and Methods:** Apoptosis of INS-1 cells was induced either by serum deprivation or by treatment with the combination of 100 U/ml Interferon-γ (IFN-γ) and 50 ng/ml tumor necrosis factor-α (TNF-α). The DNA fragmentation induced during apoptotic cell death was evaluated by the measurement of mono- and oligonucleosomes using an ELISA kit. Electrophoretic mobility shift assay was performed to detect the DNA binding of cytokine-activated transcription factors. Protein tyrosine phosphorylation was examined by immunoblotting and the expression of NO synthase (iNOS) by northern blotting. **Results:** Addition of GH or PRL decreased the amounts of oligonucleosomes during the incubation of INS-1 cells in a serum-free medium as well as those with the cytokines. The iNOS expression induced by the cytokines was also decreased by GH. GH reduced both tyrosine phosphorylation and the DNA-binding of STAT1 activated by IFN-γ, whereas the TNF-α-induced NF-κB binding was unaffected. The production of NO was, however, unlikely to be involved as the major mechanism, because its inhibition by Nω-nitro-$_L$-arginine failed to prevent the cytokine-induced apoptosis. **Conclusions:** GH and PRL could prevent the apoptotic death of insulin-secreting cells. The inhibition of IFN-γ-activated STAT1 activation may be involved in the protection from the cytokine-induced apoptosis by these hormones, although that of iNOS induction does not appear to play a major role.

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THE EFFECT OF KINASE INHIBITORS ON APOPTOSIS INDUCED BY SODIUM FLUORIDE IN RINm5F CELLS.

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Aims: It has been demonstrated previously that NaF induces apoptosis of RINm5F cells and that pretreatment with pertussis toxin (Ptx) results in an enhancement of this response. Thus, G-proteins have been implicated in the control of apoptosis. As Ptx sensitive G-proteins can regulate protein phosphorylation by modulating the activity of kinases and phophatases, the effect of tyrosine and serine/threonine kinase inhibitors on NaF and Ptx reponses was investigated. **Methods:** RINm5F cells were treated with NaF (5mM); Ptx (0.2ug/ml) and inhibitors and the extent of cell death quantified by trypan blue staining. Apoptosis was confirmed by DNA electrophoresis and acridine orange staining. **Results:** Treatment with 25uM genistein resulted in a significant reduction in NaF induced cell death (Control: 2168±280 dead cells/ml Genistein: 3409±360 NaF: 10821±666 NaF plus genistein: 7663±840 $p<0.05$). Ptx enhanced NaF induced cell death (NaF plus Ptx 16143±1846 $p<0.05$) and this effect was abolished by genistein (NaF/Ptx/genistein: 7529±858 $p<0.01$). Herbimycin A, a structurally unrelated tyrosine kinase inhibitor, also prevented the Ptx mediated enhancement of NaF induced cell death. At high concentrations (100uM) genistein alone induced apoptosis of RINm5F cells. However, this was probably mediated by inhibition of topoisomerase II as it could be blocked by pretreatment with ciprofloxacin, which competitively binds to, but does not inhibit, topoisomerase II. As Ptx also results in an increase in intracellular cAMP and activation of protein kinase A, the effect of Rp-cAMP-S, an inhibitor of protein kinase A, was investigated. Rp-cAMP-S decreased cell death in response to NaF alone but did not abolish the enhancement caused by Ptx. **Conclusion:** These results suggest that changes in both serine/threonine and tyrosine phosphorylation can regulate apoptosis in the ß-cells.

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INVESTIGATION OF THE EFFECTS OF HUMAN SERA FROM NEWLY DIAGNOSED DIABETIC PATIENTS ON CELL VIABILITY AND DEATH

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β-cell destruction *in vivo* is the result of a sustained attack by cellular and soluble factors of the immune system. Apoptosis is at least partly responsible for β-cell death *in vivo*. However, the type of β-cell death provoked by serum soluble factors *in vitro* is still open to speculation. **Aim:** To determine whether serum from newly diagnosed diabetic subjects caused DNA damage or cell death by apoptosis *in vitro*. **Methods:** Human Jurkat T cells and human islet single cells were cultured with sera from at least seven control and seven diabetic subjects. Cell membrane integrity, DNA strand breakage and chromatin condensation were recorded using cytochemical staining and single cell gel electrophoresis. **Results:** DNA damage induced by human sera (10% for 48h) was significantly dependent on Jurkat cell density in culture but was not significantly different in cells treated with diabetic (DM) sera versus control sera. The percentage of Jurkat cells which were intact and non-apoptotic was slightly but signficantly reduced by DM sera from 96.3±0.6% to 93.3±0.9% ($P<0.04$; $N=11$). Corresponding values in cytokine or etoposide treated Jurkat cells were 83.7% and 75.2% respectively. Apoptosis and necrosis levels were 1.0±0.2 and 2.1±0.4 after culture in control sera ; 1.9±0.4 and 3.1±0.9 in DM sera; 8.5±2.4 and 3.9±1.8 in cytokine-treated Jurkat cells in the same experiments. However, DM sera (10%, 48h) increased cell death by necrosis in human islet single cells from 14 to 39% (cytokine to 16%) without significant apoptosis ($P<0.05$; $N=3$). **Conclusion:** Sera from DM subjects caused a significant increase in cell death *in vitro* in Jurkat T cells and in human islet cells.

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COMPARISON OF APOPTOSIS DETERMINATION METHODS

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Results obtained using different apoptosis determination methods are dependent on factors such as treatment time, tissue culture medium additions, cell type, attachment and density. **Aim** To compare three different apoptosis determination techniques *in vitro* using a human T-cell line (Jurkat) and primary tissue (neonatal rat islets of Langerhans). **Methods** Jurkat cells were treated with a combination of interleukin-1ß(100pM), interferon-γ (200U/ml) and tumour necrosis factor-α(100pM-48h) or etoposide (10μM-18h) to induce apoptosis. Rat islets were also treated with cytokines. Cell suspensions were divided into three fractions for apoptosis determination: two fractions were placed on separate slides for 'live' fluorescent microscopic analysis by acridine orange or Hoechst 33342/propidium iodide (HPI) staining.The third fraction was fixed and placed on slides for 3'OH end labeling using the ApopTag™ kit. **Results** For Jurkat control cells ApopTag™ gave percentage apoptosis levels of 6.6±0.1, rising to 16.8±1.5 after cytokine treatment and 27.6±0.9 following etoposide treatment ($p<0.05$, N=6). Corresponding values for acridine determination were 4, 8.8 and 22.9 and by HPI were 1.7,13 and 22.5. In rat islet cells, control levels of apoptosis were 2.8±0.7, 2.1±0.6 and 1.5±0.5 (ApopTag™, acridine, HPI). Following cytokine treatment, increases in apoptosis rates varied irrespective of methodology. **Conclusions** ApopTag™ generally detected higher levels of apoptosis than either HPI or acridine. There is good correlation between results from the different methodologies especially at higher apoptosis levels. Where apoptosis rates are low, results by all of these methods are variable.

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APOPTOSIS AND THE PROTECTIVE EFFECT OF CALCIUM CHANNEL BLOCKERS ON THE NEWBORN STZ-DIABETIC RATS THYMUS

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Aims: Streptozotocin (STZ) causes an evident atrophy on the thymus and increases intracellular calcium.The increased intracellular calcium stimulates apoptosis. In this study we aimed to investigate the apoptosis on tymus tissues of STZ-diabetic newborn rats and the effect of calcium channel blockers (CCB) on the apoptosis by using in situ DNA end labelling and DNA ladder methods showing DNA fragmentation.
Material and Methods: On the second day of the birth 100 mg/kg STZ was given i.p. to the first two groups of the newborn Wistar rats. 1st group was STZ diabetic. To the 2nd group , starting from 12th week, 5mg/kg/day isradipine (i.p) was given for 6 weeks. To the 3rd group, same dose isradipine was given on the 2nd day than STZ was given on the 3rd day of the birth. The 4th group was nondiabetic and treated 5mg/kg/day isradipine at the same period. The 5th group was the control. We were used the dexametasone treated rats for the positive apoptosis control group. The thymus tissue samples were fixed in 10 % neutral buffered formalin and embedded in paraffin then applied in situ DNA end labelling method for detection of apoptotic cells. The DNAs were isolated from fresh thymus tissues and DNA ladder method was applied on gel electrophoresis for DNA fragmentation.
Results: According to the results of DNA fragmentation and the dexametasone treated group (15±5.1) as positive control, more labelled apoptotic cells were observed in thymus of isradipine (14.5±7.0), STZ (9.5±2.8) and STZ+isradipine treated groups (7.4±1.9) when compared with the other groups (control;5.0±1.8, isradipine + STZ; 6.2±2.0).
Conclusions: Our data show that isradipine as a CCB has a protective effect on apoptosis in the newborn STZ- diabetic rat thymus.

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DIFFERENTIAL ROLE OF GLUT2 GLUCOSE TRANSPORTER IN THE SELECTIVE β-CELL TOXICITY OF ALLOXAN AND STREPTOZOTOCIN

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Aims: The diabetogenic agents alloxan and streptozotocin are widely used in experimental diabetes research due to their specific β-cell toxicity. Both molecules exhibit a marked structural similarity to glucose. Thus the hexose binding structures glucokinase and the GLUT2 glucose transporter are potential targets or a crucial transporter of the toxins. It was the aim of this study to investigate the specific function of the GLUT2 glucose transporter for the toxicity of alloxan and streptozotocin. **Methods:** The GLUT2 glucose transporter was stably overexpressed in the insulinoma cell line RINm5F. After exposure to alloxan or streptozotocin cell viability was measured by the MTT assay. The function of the GLUT1 and the GLUT2 glucose transporter isoforms was quantified by 3-OMG uptake kinetics. GLUT2 expression was determined by Northern and Western blot analyses. **Results:** RINm5F control cells, which showed a very low expression level of the GLUT2 glucose transporter compared to normal β-cells, were resistant to alloxan and streptozotocin. Through overexpression of the GLUT2 glucose transporter RINm5F cells became sensitive to alloxan and streptozotocin with IC_{50} values of 10 mM and 1.4 mM, respectively ($p < 0.01$). Glucose provided a significant protection of RINm5F-GLUT2 cells against the toxicity of alloxan but not against streptozotocin. Alloxan (15 mM) selectively inhibited the GLUT2 transport function of RINm5F-GLUT2 cells by more than 50 % ($p < 0.01$). The expression level of the GLUT2 glucose transporter protein was neither affected by exposure to alloxan nor to streptozotocin. **Conclusion:** The GLUT2 glucose transporter is of differential importance for the selective ß-cell toxicity of alloxan and streptozotocin. While the sensitivity of RINm5F-GLUT2 cells to streptozotocin can be explained by an increased transport capacity for this toxin, the GLUT2 transporter protein itself seems to be a target structure for the toxicity of alloxan.

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THE INFLUENCE OF LOW DOSE CHRONIC RADIATION ON GLUCOSE TOLERANCE IN RATS

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The aim of study was the investigation of the development of the glucose tolerance disturbance in rats under natural conditions of Chernobyl Exclusion Zone. **Materials and methods.** 3 groups of 3 month-old Wistar male rats were exposed to radiation by incorporated radionuclides (in the main $^{137+134}Cs$ and ^{90}Sr from contaminated food and water) and external γ-background in hutches (40-60 μR/h) during 12 months. The 4-th group of rats was the control one, which was obtained clean forage and water. γ-Background was 12-15 μR/h in their hutches. After 1,5 months the total absorbed doses (TAD) in 3 groups were 0.2, 0.7 and 7.3 cGy and after 12 months - 1.7, 5.0 and 50.8 cGy. Blood glucose levels were tested by glucose-oxidaze method during GTT (2 g glucose on kg, i.p.) in the above periods. **Results.** The rats with TAD 7.3 cGy during 1,5 months showed higher fasting glucose levels (5,9 ± 0,4 vs 3,7±0,2 in control group, $p<0.01$) and «plane» glycaemic pattern. Irradiation in TAD 0.2 and 0.7 cGy did not change of glucose tolerance in rats. After prolonged irradiation (12 months) there were no differences in fasting glucose levels between experimental and control groups. However TAD 50.8 cGy provoked the disturbance of glucose tolerance: glycemia did not return to basal level at 120 min after glucose load (4.8 ± 0.4 vs 3.5±0.4 mmol/l, $p<0.05$) and integral glycemia level over GTT were 23.9 ± 2.9 vs 17.7 ± 0.3 mmol/l in control group ($p<0.05$). We **conclude** that highest radiation load induces the glucose tolerance modification in both periods of investigation, however glucose tolerance disturbance in rats after long-term radiation exposure is obviously.

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Beta-Cell Function

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REDUCED B-CELL COMPENSATION TO THE INSULIN RESISTANCE OF AGING: IMPACT ON PROINSULIN AND INSULIN LEVELS.

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Aging and Type 2 diabetes mellitus are both associated with insulin resistance and reduced B-cell function. In Type 2 diabetes this reduction in B-cell function is associated with an increase in the proportion of immunoreactive insulin (IRI) comprised of proinsulin (PI; increased PI/IRI ratio).

To determine whether the alteration in B-cell function and insulin sensitivity of aging is associated with an increased PI/IRI, we studied 27 healthy older (age: 67±1yr, mean±sem) and 22 younger (28±1 yr) subjects with comparable degree of body adiposity (BMI: 27.9±0.6 vs. 26.3±1.0 kg/m^2). PI was measured by a RIA recognizing both intact PI and its conversion intermediates. The insulin sensitivity index (S_I) was quantified using Bergman's minimal model and B-cell function was measured as the acute response to glucose (AIRglucose) and as the acute response to arginine at maximal glycemic potentiation (AIRmax). Measures of B-cell function was also adjusted for S_I based on the known hyperbolic relationship between these two variables (S_I x AIRglucose and S_I x AIRmax).

Older and younger subjects had similar fasting glucose (5.4±0.1 vs. 5.2±0.1 mmol/l), IRI (82±8 vs 76±9 pmol/l), PI (8.8±0.8 vs. 10.6±2.0 pmol/l) and PI/IRI (12.4±1.2 vs. 13 9±1.6%)(all p=NS). This was despite a 50% reduction of insulin sensitivity (S_I: 1.96±0 21 vs. 3.88±0.38 x 10^{-5} min^{-1}/pmol/l, p<0.001) and in B-cell function (S_I x AIRglucose: 0.72±0.13 vs. 1.70±0.15 x 10^{-2} min^{-1}, p<0.001; S_I x AIRmax: 3.53±0.52 vs. 6.81±0.70 x 10^{-2} min^{-1}, p<0.001) in the older subjects.

In conclusion the aging-associated reduction in B-cell function does not include disproportionate proinsulinemia. Thus, the normal aging process and Type 2 diabetes appear to be distinct entities. Fasting levels of insulin and its precursors as indicators of insulin resistance in aging may not be comparable with indices in younger subjects.

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BETA CELL FUNCTION IN THE CONTEXT OF INSULIN SENSITIVITY IN OBESE PATIENTS

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It is known that obese persons are insulin resistant, what can contribute to the development of impaired glucose tolerance or insulin-nondependent diabetes mellitus. Normal glucose homeostasis is maintained as long as insulin secretion compensatory increases, accordingly beta-cell function should be assessed in the context of peripheral insulin sensitivity (so called "disposition index"). **Aim:** Aim of our study was to evaluate insulin sensitivity, acute insulin response to glucose and disposition index in obese patients. **Materials and methods:** Ten obese patients (6 female, 4 male; age: 28.16+/-1.3 yr; BMI: 31.29+/-1.15 kg/m^2) were studied. Control group was age and sex matched, but not obese (5 female, 5 male; 30+/-1.28 yr; BMI: 25.12+/-1.01 kg/m^2). Both obese patients and control group had normal glucose tolerance during OGTT. Insulin sensitivity was estimated by MINMOD method (R.Bergman) of frequently sampled IVGTT (FSIGT). Acute insulin response to glucose (AIR_G) was calculated as the mean increment above basal of insulin values measured at 2-10 min during FSIGT (0.3 g/kg i.v. as 50% glucose) and disposition index as the product of AIR_G and insulin sensitivity. **Results:** Decreased insulin sensitivity was found in the tested patients compared to the control group (1.97+/-0.3 x 10^{-4} vs. 4.81+/-0.7 x 10^{-4} min/mU/l, $p < 0.01$). No significant difference was found in acute insulin response (68.35+/-20.12 vs 56.22+/-19.05 mU/l, $p > 0.05$) between obese patients and non-obese control group. However, disposition index was significantly decreased in obese persons in comparison to non-obese control group (136.32+/-14.5 x 10^{-4} vs. 265.34+/-10.36 x 10^{-4}, $p < 0.01$). **Conclusion:** In conclusion, acute insulin response though normal was not adequate for the degree of insulin resistance in the tested obese patients. Assessment of insulin secretion in the context of insulin sensitivity early demonstrated defect in beta-cell function that could not be detected if only insulin secretion was measured.

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LACK OF PRIMING EFFECT OF PULSATILE VERSUS CONTINUOUS INSULIN ON INSULIN SENSITIVITY IN MAN

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Aims: Insulin is secreted in man in regular pulses every 8 to 13 minutes. Disordered pulsation has been demonstrated in several insulin resistant states. It is not clear whether this represents a primary beta cell defect contributing to insulin resistance or is a consequence of insulin resistance. Basal or near basal insulin by pulsatile infusion augments hypoglycaemic effect and improves insulin mediated glucose uptake compared with insulin by continuous infusion. No study has examined if normal basal insulin pulsatility is required to preserve subsequent insulin sensitivity during hyperinsulinaemia. We studied the effect of pulsatile or continuous insulin at basal levels overnight on a subsequent hyperinsulinaemic euglycaemic clamp. **Materials and Methods:** We studied 8 normal male volunteers (mean age 21.3 years, range 18-33) on 2 occasions each, administering 5.4 mU kg^{-1} h^{-1} of insulin by continuous infusion for 8h or in pulses of 2 mins every 13 mins for 8h. Endogenous insulin secretion was inhibited by octreotide at 0.43 μg kg^{-1} h^{-1} (plasma C peptide levels < 1.0 g/l within 2h of commencing the octreotide infusion). Glucagon was replaced by a continuous infusion at basal levels (30 ng kg^{-1} h^{-1}). After discontinuing overnight insulin peripheral insulin action was assessed by a hyperinsulinaemic euglycaemic clamp (1 mU kg^{-1} h^{-1}). **Results:** Insulin pulsation was confirmed by measurement of plasma insulin just prior to and 3 minutes after each pulse. Plasma glucose measured hourly throughout the night showed a trend for lower glucose on the pulsatile night but was not significantly different. There was no difference in glucose infusion rates in the final 30 mins of the euglycaemic clamp between the pulsatile and continuous infusion. **Conclusion:** Pulsatile compared with continuous insulin administration has no significant priming effect on subsequent peripheral insulin mediated glucose uptake.

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A MECHANISM FOR INSULIN RESISTANCE IN PREGNANCY? A STUDY OF INSULIN KINETICS.

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The mechanisms of insulin resistance in pregnancy are poorly understood. Marked differences in peak plasma insulin concentration (Ip) after insulin modification of a frequently sampled intravenous glucose-tolerance-test (glucose 0.3g/kg) were observed in a study investigating mechanisms of fetal nutrition. **Aim:** To investigate whether the differences in insulin distribution volume or elimination rate (ki) between subjects were the cause of the different Ip. **Materials and Methods:** Twenty-seven women at 34 weeks gestation were studied. The apparent volume of distribution (Vd) and ki were quantified by fitting the regression line to the log of the insulin concentrations and deriving Vd from the insulin dose (0.02U/kg) and the insulin concentration extrapolated to time zero and ki from the slope of the regression line. Specific insulin was assayed using an ELISA. The Ciba program was used to derive Si using the minimal model and the Homeostasis Model Assessment programme was used to derive %S. **Results:** The median (IQR) Si = 8.7 (6.6 – 19.5) (l/(min.pmol/l)) x10^{-5}, %S = 141.6 (91.7 – 207.7) %, ki = -0.059(-0.082 - -0.052) min^{-1}, clearance = 43.7 (2.7 – 6.4) l/min and Vd = 59.3 (43.5 – 106.1) l. There were significant correlations between Vd and ln Si r=0.73 (p<0.0001), Vd and %S r=0.48, p=0.01, ki and ln Si r=0.44, p=0.02, ki and ln %S r=0.66, p<0.0001, clearance and ln Si r=0.79, p<0.0001, and clearance and ln %S r=0.67, p<0.0001. **Conclusions:** These data demonstrate that ln Si and ln %S ∝ Vd, ki and clearance over a range of Si and %S. We hypothesise that the correlation of insulin kinetics with insulin mediated glucose disposal is due to impaired insulin stimulated endothelium mediated vasodilatation and reduced trans-endothelial transport of insulin. This may have implications for our understanding of the mechanisms of peripheral insulin resistance in pregnancy.

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INSULIN RESISTANCE IS THE PREDOMINANT FEATURE IN NORMOGLYCAEMIC WOMEN WITH PREVIOUS GESTATIONAL DIABETES

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Aims: Women with previous gestational diabetes (GDM) are at high risk of type 2 diabetes in later life. Clinical investigation of such women should allow early defects in the evolution of type 2 diabetes to be defined. Materials and methods: We studied a total of 89 normoglycaemic European women, 39 with a history of previous GDM and 50 control women (matched for age, parity and time since delivery) who had had normal pregnancies. Estimates of β-cell function (%B) and insulin sensitivity (%S) were derived from fasting glucose and insulin measures using the HOMA algorithm. In a subgroup of 20 GDM and 23 control women, insulin sensitivity (S_I), glucose effectiveness (S_g) and insulin secretion were estimated by minimal modelling of an insulin-modified intravenous glucose tolerance test (IVGTT). Results: Compared to controls, GDM women displayed marked insulin resistance as evidenced by: lower HOMA%S (median (IQR), 23(18-47) vs 95(62-167)%, $P<0.0001$), higher fasting insulin (206(96-267) vs 48(28-76)pmol/l, $P<0.0001$), hypertriglyceridaemia (1.1(0.8-1.3) vs 0.8(0.6-1.1) mmol/l, $P=0.02$), increased WHR ((mean±SD) 0.81±0.07 vs 0.77±0.06, $P=0.01$) and, in the IVGTT group, lower S_I (0.7(0.3-1.0) vs 1.4(1.2-2.0)10^{-4}/min/pmol/l, $P=0.001$) and reduced NEFA suppression (19 min: 292±133 vs 212±110 μmol/l, $P=0.03$). Meanwhile, β-cell function was maintained as shown by: higher HOMA%B (238(141-349) vs 99(70-137)%, $P<0.0001$), similar proinsulin:insulin ratios (0.04(0.03-0.08) vs 0.05(0.02-0.12), $P=0.7$) and incremental 1st phase insulin (2-10 mins) (967(418-2340) vs 1005(423-1458) pmol/l/h, $P=0.2$). BMI and fasting leptin levels were similar in the 2 groups. Conclusions: Insulin resistance is the dominant metabolic feature in normoglycaemic European women with previous GDM. Preservation of β-cell function allows these women to maintain normoglycaemia despite this marked insulin insensitivity.

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STRESS COMBINED WITH GESTATIONAL DIABETES INDUCES GLUCOSE TOLERANCE AND LIPID PEROXIDATION DISTURBANCES IN RATS SECOND GENERATION FEMALE OFFSPRING

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Aim: To evaluate the vertical transmission of maternal social stress (MSS) against the background of gestational diabetes (GD)-induced glucose intolerance in the first generation female offspring (F_1) to the second generation female offspring (F_2) and to characterise the oxidation parameters in the above. **Materials and Methods:** For MSS creation Wistar rats were transferred daily from one rats association to another within 2^{nd} – 8^{th} day of pregnancy and GD was rendered by STZ injection (45 mg/kg, i.p.) on the second day of pregnancy (n=8). I.p. GTT (3g glucose/kg; 0, 30, 60 and 120 min) was performed in the female offspring F_1 (n=10), F_2 (n=16) and in controls (n=25) at 45 and 90 days of age. Diene conjugates (DC), malonic dialdehide (MDA), reduced glutathione (GSH) levels and catalase activity (CA) in liver were determined spectrophotometrically in the same age. **Results:** The impairment of glucose tolerance (GT) was shown in F_1 before mating as compared to controls ($p<0.05$). It was revealed significant GT decrease in F_2 at 45 days of age (integral glycemia over i.p. GTT: 30.0±0.8 vs 24.2±2.0 mmol/l in controls, $p<0.01$) and at 90 days of age (integral glycemia over i.p. GTT: 31.5±2.4 vs 25.9±1.5 mmol/l in controls, $p<0.05$). Liver DC and MDA levels were increased in F_2 at 90 days of age (DC: 106.3±8.6 vs 80.4±9.3 μmol/g in controls, $p<0.05$; MDA: 403.3±45.8 vs 84.4±2.7 μmol/g in controls, $p<0.001$), whereas CA did not change and GSH levels were decreased by 30 % ($p<0.01$) only in F_2 at 45 days of age. **Conclusion:** Maternal social stress against the background of gestational diabetes induced glucose tolerance impairment in F_2 female rats offspring beginning from the puberty and strengthened lipid peroxidation at the sex-maturity period.

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EFFECTS OF AZELAIC, SEBACIC AND DODECANOIC ACIDS ON INSULIN RELEASE

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Aims: The utilization of medium-chain dicarboxylic acids, such as azelaic, sebacic and dodecanoic acids, as alternative fuel substrate in parenteral nutrition was recently proposed. In the present study, the effects of these water-soluble nutrients and one of their esters on glucose-stimulated insulin release were investigated in rat pancreatic islets. **Materials and methods:** For measuring insulin release, groups of 8 islets each isolated from fed Wistar rats were incubated for 90 min at 37°C in the presence of D-glucose (7.0 or 11.1 mmol/l) and, as required, azelaic acid (10.0 mmol/l), sebacic acid (10.0 mmol/l), dodecanoic acid (10 mmol/l) or glycerol-1,2,3-tris(dodecanoate) (4.0 mmol/l). Control experiments indicated that the dicarboxylic acids failed to interfere with the immunoassay of insulin. Glycerol-1,2,3-tris(dodecanoate), however, increased ^{125}I-insulin binding to guinea pig anti-insulin antibodies, without affecting the blank value (no antibody) or the partial neutralization of the antibodies by secreted insulin. **Results:** At 7.0 mmol/l D-glucose, both azelaic and sebacid acid augmented significantly insulin output. On the contrary, dodecanoic acid and glycerol-1,2,3-tris(dodecanoate) decreased glucose-stimulated insulin output. Essentially comparable results were recorded in islets incubated at a higher concentration of D-glucose (11.1 mmol/l). **Conclusions:** The present results extend to insulin-producing cells the knowledge that medium-chain dicarboxylic acids, such as azelaic and sebacid acids, might be regarded as useful energy substrates. Such was apparently not the case, however, in islet cells exposed to dodecanoic acid and its triglyceride ester. These findings are obviously relevant to the use of these alternative nutrients in clinical situations such as decompensated diabetes mellitus.

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In Vivo Metabolism

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INFLUENCE OF NUTRITIONAL STATUS ON THE PATHWAYS OF GLUCOSE DISPOSAL DURING AN OGTT IN NORMAL SUBJECTS

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Aims: To assess the influence of nutritional status on the fate of oral glucose in normal subjects. **Materials and Methods**: Six normal subjects were submitted to three 5-h OGGTs performed in random order after an overnight fast (ONF), a 4-day fast and an overnight i.v. glucose infusion (4 mg/kg.min for 11h, discontinued 2h before the OGTT) to simulate a "fed" state. The 75 g glucose were labelled with 3-^{3}H- and U-^{14}C-glucose to measure glycolysis ($^{3}H_2O$ production), glycogen synthesis (uptake - glycolysis), glucose oxidation (ox) ($^{14}CO_2$ output) and nonox glycolysis, in combination with indirect calorimetry. **Results:** After the ONF, oral glucose disposal over 5h was as follows: uptake : 70±1g; glycolysis : 40±2g; glycogen synthesis : 30±2g; ox : 17±1g and nonox glycolysis : 23±1g. Total carbohydrate (CHO) ox (indirect calorimetry) averaged 37±3g. None of the dietary manipulations affected glucose uptake. Compared with the ONF, the 4-day fast inhibited glycolysis (-24±3 %; P<0.001) in relation to a 41±6% (P<0.001) decrease in ox and stimulated but not significantly glycogen synthesis (+22±10%; NS). Total CHO ox decreased by 87±3% (P<0.001) and net CHO balance (uptake - CHO ox) increased by 94±13% (P<0.001). In contrast, prior "feeding" stimulated glycolysis (+21±5%; P<0.01) but not glucose ox and inhibited glycogen synthesis (-33±3 %; P<0.001). CHO ox increased by 41±8% (P<0.005) and net CHO balance was reduced by 50±8% (P<0.005). **Conclusions:** The nutritional status influences the metabolic fate of an oral glucose load including its rate of conversion to glycogen. However, because the absolute changes in total CHO balance are far greater than those in glycogen synthesis, it is suggested that the net storage of oral glucose is mainly regulated by the rate of glycogen oxidation.

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CHANGES IN BLOOD INSULIN LEVEL DURING ORAL LIPID LOAD AND OGTT IN OBESE AND NON-OBESE WOMEN WITH POLYCYSTIC OVARY SYNDROME.

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The polycystic ovary syndrome (PCOS) is known to be associated with several metabolic abnormalities among which an insulin resistance is of particular interest. **The aim:** of the present study was to estimate the insulin response to the oral lipid or carbohydrate load (during OLTT and OGTT respectively) in obese and non-obese women with PCOS in comparison with that in healthy women. **Materials and Methods:** All the data are expressed as mean±S.E.M. Four groups were studied: 1 non-obese women without PCOS, n=17, age 22,12±0,58 y.o., BMI=20,51±0,32 kg/m^2, WHR=0,69±0,01, they had regular ovulatory cycles; 2 obese women without PCOS, n=6, age 22,83±2,46 y.o., BMI=29,10±2,42 kg/m^2, WHR=0,76±0,40, all the women had ovulatory cycles; 3 non-obese women with PCOS, n=13, age 22,31±0,94 y.o., BMI=20,17±0,56 kg/m^2, WHR=0,69±0,01; 4 obese women with PCOS, n=9, age 25,11±1,77 y.o., BMI=33,32±2,35 kg/m^2, WHR=0,79±0,26. All the subjects underwent a standard glucose tolerance test (OGTT) on day 9 of a menstrual cycle and an oral lipid tolerance test (OLTT) with the lipid load of sour cream (20% fat) in a dose 130 g/2 m^2 of body square) on day 20-22 of a menstrual cycle. Statistical analysis was performed using Student's t-test. **Results:** Serum insulin levels during OLTT at 0, 3, 9 and 24 hours respectively: Group 1: 7,49±0,55, 12,15±1,54, 6,31±0,49, 5,03±0,40 μU/l; Group 2: 12,84±4,45, 19,27±8,66, 8,57±1,58, 11,60±5,59; Group 3: 6,49±0,56, 8,77±0,84, 5,88±0,64, 4,70±0,40; Group 4: 9,41±3,03, 12,38±3,13, 7,17±1,75, 8,77±2,39 μU/l. Serum insulin levels during OGTT at 0, 30, 60 and 120 min respectively: Group 1: 6,85±0,59, 41,47±4,63, 36,81±6,69, 17,84±4,41 μU/l; Group 2: 16,25±6,63, 102,49±33,16, 82,56±26,50, 53,69±31,67 μU/l; Group 3: 6,03±0,90, 46,78±6,21, 40,53±6,73, 23,48±3,67; Group 4: 13,25±4,09, 57,85±15,44, 67,63±15,88, 54,26±16,67 μU/l. **Conclusion:** there is no significant difference in the levels of insulin during both OGTT and OLTT between obese and non-obese women with PCOS.

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MECHANISMS BY WHICH HYPERGLYCAEMIA *VS* HYPERINSULINAEMIA STIMULATE GLUCOSE STORAGE IN FASTED SUBJECTS

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Aims: It is known that prior fasting stimulates glucose storage on refeeding. This study aims at determining whether hyperglycaemia *vs* hyperinsulinaemia exert this effect by identical mechanisms. **Materials and Methods:** Two groups (I and II) of 7 normal volunteers were fasted for 4 days and clamped for 4h at a glucose level of 155 ± 15 mg/dl and an insulin level of 106 ± 5 μU/ml under somatostatin infusion in group I and at 86 ± 3 mg/dl and 2800 ± 100 μU/ml in group II. Constant infusion of 3-^{3}H and U-^{14}C-glucose allowed to measure glucose uptake (Rd), glycolysis ($^{3}H_2O$ production), glycogen synthesis by the direct pathway (Rd - glycolysis), glucose oxidation (ox) ($^{14}CO_2$ output), nonox glycolysis (glycolysis - ox) and nonox glucose disposal (NOGD) (Rd - ox). The latter includes both the direct and indirect pathways of glycogen storage. **Results:** Similar Rds were obtained in groups I and II (305 ± 17 *vs* 281 ± 24 mg/m².min; NS) during the last h of the clamps. Pathways of disposal, expressed in % of Rd were the following. Glycolysis : 32 ± 2 *vs* 43 ± 2 (P < 0.005); glycogen synthesis : 68 ± 2 *vs* 57 ± 2 (P < 0.005); ox : 21 ± 1 *vs* 20 ± 1 (NS); nonox glycolysis : 12 ± 1 *vs* 24 ± 12 (P < 0.005) and NOGD : 79 ± 1 *vs* 80 ± 1 (NS). The steady state lactate levels which correlated strongly with nonox glycolysis (r = 0.85; P<0.001) were lower in group I *vs* II (1.32 ± 0.06 *vs* 1.79 ± 0.12 mM; P < 0.005). Energy expenditure (indirect calorimetry) averaged 869 ± 22 *vs* 958 ± 23 kcal/m².24h (P < 0.02). **Conclusions:** At similar Rds, hyperglycaemia and hyperinsulinaemia lead to identical overall glycogen synthesis in fasted subjects but hyperglycaemia is associated with a higher ratio of direct/indirect pathway and with a lower energy cost of storage.

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BODY MASS INDEX AND BASAL TRIACYLGLYCEROL CONCENTRATIONS INFLUENCE GLUCOSE EFFECTIVENESS IN ADULT MEN

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Aims:
To explore relationships between glucose and lipid metabolism; specifically those between fasting concentrations of non-esterified fatty acids (NEFA), triacylglycerol (TAG) and indices of glucose disposal i.e. insulin sensitivity (S*I) and glucose effectiveness (S*G).

Materials and Methods:
14 healthy non-obese men aged 38.5±4.1 y of body mass index (BMI) 25±2 kg.m^{-2} with normal fasting plasma glucose (5.27 ± 0.30 mmol.l^{-1}) were studied. Fasting concentrations of NEFA and TAG were measured. All subjects underwent a stable-label IVGTT in the fasting state. Indices of S*I and S*G were obtained using the MINMOD computer program.

Results:
Fasting NEFA concentrations averaged 279 ± 31(SE) mmol.l^{-1}. Fasting TAG concentrations were 1.16±0.39(SE) mmol.l^{-1}. S*G was 0.0111±0.0009 min^{-1}; S*I was 6.44±1.21(SE), 10^4.min^{-1}/μUml^{-1}. Basal TAG concentrations showed a significant inverse correlation with S*G (r=-0.73, ***p=0.001***) but not with S*I (r=-0.30, p=0.31). BMI also correlated inversely with S*G (r=-0.62, ***p=0.02***) but not with S*I (r=-0.44, p=0.11). NEFA concentrations showed no significant relationships.

Conclusions:
The data suggests that the influence of triacylglycerol and BMI on glucose disposal may be mediated by glucose effectiveness rather than insulin sensitivity.

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SPLANCHNIC GLUCOSE UPTAKE FOLLOWING ORAL GLUCOSE ADMINISTRATION IN TYPE-1 DIABETES

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Aims: Splanchnic glucose uptake (SGU) is a major determinant of postprandial glycemia and thus glucose control in patients with diabetes. While SGU is decreased in Type-2 diabetes, there is considerable uncertainty whether SGU is altered in Type-1 diabetes. We therefore intended to study SGU in patients with Type-1 diabetes compared to healthy controls. **Methods:** To this end a recently developed and validated non invasive method (OG-CLAMP) determining glucose disposal and SGU by combining a hyperinsulinemic (120 mU/m^2/min) euglycemic clamp and an oral glucose load (75 gms.) was employed during steady-state of glucose disposal. Six patients with Type-1 diabetes (D, all male, age 34±3 (x±SEM) years, BMI 25±2.2 kg/m^2, HbA1c 7.8±0.8%) and matched, healthy controls (C) underwent such OG-CLAMP after an overnight fast. **Results:** Glucose disposal rate was 10.1±0.5 mg/kg/min in C, and 9.7±1.0 in D (p=0.7, C vs. D), the time required for resorption of the glucose load was 131±5 min in C, and 147±7 min in D (p=0.1, C vs. D). SGU was not different between C (13.4±3.1%) and D (8.5±2.4%, p=0.25). **Conclusion:** Unlike in Type-2 diabetes, splanchnic glucose uptake is not altered in insulin sensitive euglycemic patients with Type-1 diabetes. These findings argue against an influence of protracted prior hyperglycemia on the amount of orally administered glucose taken up by the liver.

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ANOMERIC SPECIFICITY OF HUMAN B-CELL GLUCOKINASE: MODULATION BY ITS REGULATORY PROTEIN

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Aims: The role of glucokinase in the anomeric specificity of D-glucose-stimulated insulin release remains controversial. This issue was reinvestigated using recombinant human liver and B-cell glucokinase. **Methods:** In the absence of its regulatory protein, the anomeric specificity of glucokinase was examined over 10 min incubation at 20-22°C in the presence of 1.0 mmol/l ATP and 2.5 to 50 mmol/l α- or ß-D-glucose, mixed with a tracer amount of the corresponding D-[U-^{14}C]glucose anomer. The influence of the regulatory protein was examined in experiments conducted over 6 min incubation at 30°C, the concentration of the regulatory protein being selected to decrease the reaction velocity by about half at low concentrations of D-glucose and in the presence of 0.2 mmol/l D-fructose 6-phosphate. **Results:** Liver glucokinase displayed a lower maximal velocity, Hill number and apparent Km with α- than ß-D-glucose. As a result of these differences, the reaction velocity was significantly higher with α- than ß-D-glucose up to 10 mmol/l. Islet glucokinase also displayed a lower maximal velocity and apparent Km for α- than ß-D-glucose, but no obvious difference in Hill number. The reaction velocity failed to be significantly higher with α- than ß-D-glucose even at low hexose concentrations. The anomeric specificity of islet glucokinase was little affected by its regulatory protein. The residual reaction velocity, when expressed relative to the paired control value (no regulatory protein), averaged in the case of ß-D-glucose 95.9 ± 4.9 % of the paired value found with α-D-glucose. Likewise, in the case of liver glucokinase, such a percentage averaged 93.1 ± 5.9 %. **Conclusions:** These findings support the view that, at close-to-physiological concentrations of D-glucose, the α-anomeric preference of insulin release cannot be fully accounted for by the intrinsic properties of B-cell glucokinase.

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COUNTERREGULATORY RESPONSES TO HYPOGLYCEMIA IN PATIENTS WITH GLUCOKINASE GENE MUTATIONS (MODY2).

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Aims: glucokinase gene is expressed not only in pancreatic β cells, but also in pancreatic α cells, in liver and some neuroendocrine cells in the central nervous system. A decreased glucokinase activity in the latter cell types may be expected to cause increased glucose production and a higher threshold for the counterregulatory responses to hypoglycemia. Methods: counterregulatory hormones secretion and glucose production (6,6 ^{2}H-glucose) were monitored during progressive hypoglycemia (hyperinsulinemic clamp at ca 2.4 pmol/kg/min insulin and decreasing plasma glucose concentrations from basal values to 3.3 mmol/l by steps of 0.6 mmol/l) in 7 MODY2 patients with characterized glucokinase gene mutations and 13 healthy controls. Results: counterregulatory hormones concentrations, insulin sensitivity, and insulin-induced suppression of glucose production at basal glucose concentrations were identical in MODY2 patients and healthy controls, but the curves linking glycemia to glucose production and to glucagon concentrations were switched to the right in MODY2 patients. The glycemic thresholds of MODY2 patients for glucose production (5.0±0.4 mmol/l) and for glucagon stimulation (4.5±0.4 mmol/l) were increased compared to healthy subjects (3.9±0.1 for glucose production and 3.7±0.1 for glucagon, p<0.02 in both cases). Conclusions: these data indicate that counterregulatory responses aimed at increasing plasma glucose concentration are activated at a higher plasma glucose concentration in MODY2 patients.This may be secondary to decreased glucokinase activity in both a) central nervous cells and pancreatic α cells, leading to enhanced sensitivity of counterregulatory hormones secretion to hypoglycemia, and b) liver cells, leading to an autoregulation of glucose production set at a higher level of glycemia. These consequences of decreased glucokinase activity outside pancreatic β cells are likely to contribute to the pathogenesis of hyperglycemia in MODY2 patients.

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Effect of Long-term Dietary Protein Intake on Glucose Metabolism in Man

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Abstract

A meal rich in protein stimulates insulin secretion, since it is required for postprandial amino acid disposition. As long-term effects of dietary protein on insulin secretory capacity and glucose metabolism are still unknown our study focussed on this aspect. Subjects with constant protein intake of 1.87 ± 0.26 g kg-1 day-1 (1.25 - 2.41), named high protein (HP) group, and with 0.74 ± 0.08 (0.57 - 0.80), normal protein (NP) group, were identified by a food questionnaire and were matched (n=9) according to sex, age, and calorie intake. They underwent an intravenous glucose tolerance test and a euglycemic hyperinsulinemic clamp with infusion of [6,6-2H]-glucose combined with calorimetry. To estimate net gluconeogenesis the usual diet was enriched by U-[13C]-glucose for one week, and breath and plasma were sampled on a daily basis. Glucose-stimulated insulin secretion was increased in the HP group (516 ± 45 pmol/l vs. 305 ± 32, p=0.012) due to reduced glucose threshold of the endocrine β-cells (76 ± 9 mg/dl vs. 89 ± 5, p=0.031). Hepatic glucose output was elevated by 12% (p=0.009) at 40 pmol/l plasma insulin in the HP group, but not at higher insulin levels, while overall glucose disposal was reduced. Fasting plasma glucagon was 34% increased in the HP group (p=0.038). Plasma 13C-glucose enrichment increased when the 13C-enriched diet was started and reached a plateau after four days. In NP subjects plasma U-[13C]-enrichment represented 51.5 ± 2.9% of the enrichment of hepatic glycogen. Fractional gluconeogenesis was increased by 40% (p=0.044) in individuals on a high protein diet. We conclude, that a high protein diet is accompanied by increased stimulation of glucagon and insulin within the endocrine pancreas associated with high glycogen turnover and gluconeogenesis.

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CHANGES IN WHOLE BODY PROTEIN METABOLISM DURING HYPOTHYROIDISM. INTERACTION WITH INSULIN

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Aims :We have investigated the effect of hypothyroidism and insulin on protein metabolism in humans. Materials and methods : Six hypothyroid patients were studied in a postabsorptive state before and after 5 months of regular treatment for hypothyroidism (153 ± 17 µg /day of L-thyroxine). The effect of insulin was assessed under hyperinsulinemic euglycemic and eukaliemic conditions. Insulin was infused for 140 min at 6.3 ± 0.2 nmol.kg^{-1}min^{-1}. An amino acid infusion was used to blunt insulin-induced hypoaminoacidemia. Whole body protein turnover was measured using L-[1-^{13}C] leucine. Results : When compared to L-T4 induced euthyroidism, hypothyroidism induced a significant decrease ($P< 0.05$) in leucine endogenous appearance rate (a reflection of proteolysis; 1.03 ± 0.11 vs 1.37 ± 0.06), oxidation (0.18 ± 0.002 vs 0.25 ± 0.03) and nonoxidative disposal (a reflection of protein synthesis; 1.07 ± 0.12 vs 1.36 ± 0.04). Insulin lowered proteolysis during both the euthyroid and hypothyroid states. Hypothyroidism impaired insulin's antiproteolytic effects, when expressed in absolute terms or relative to insulinemia. Conclusion : The weak effect of insulin during hypothyroidism is of pathophysiologic interest since it contrasts with an enhanced effect of insulin on protein breakdown observed in hyperthyroidism.

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Hepatic Glucose Production

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PRANDIAL GLUCOSE EFFECTIVENESS AND GLUCONEOGENESIS IN INSULIN RESISTANT RELATIVES OF PATIENTS WITH TYPE 2 DIABETES MELLITUS.

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Impaired insulin sensitivity is widely recognized in healthy relatives of type 2 diabetic individuals. In contrast, glucose effectiveness (GE) has been reported increased, normal or decreased employing the minimal model. In addition, aspects of endogenous glucose handling are not well characterized in these potential prediabetic subjects. To explore gluconeogenesis (Gn) and GE in further detail, the latter when applying a more physiological approach, 23 insulin resistant first-degree relatives (R) (M-value; P<0.05) and 10 matched controls (C) were examined using a combined somatostatin/insulin infusion (0.17 vs 0.14 mU/kg/min, R vs C) to maintain insulin concentration at basal level and a hot-ginf infusion to reproduce a prandial plasma glucose profile. Using this novel approach, the resulting glycemic profile is a direct measure of GE. Fasting rates of Gn measured using the ^2H_2O technique (7.6±0.7 vs 6.7±0.4 mmol/kg/min; P=0.29) and endogenous glucose production (EGP) (13.5±0.5 vs. 13.4±0.3 mmol/kg/min; P=0.84) did not differ between groups though fasting insulin tended to be elevated in R (28±4 vs. 41±1 pM; P=0.08). Following glucose infusion, the integrated area above basal of the glycemic profile (P=0.87) and the rate of suppression of EGP (P=0.27) were comparable. Cold (Sg) (2.61±0.25 vs. 2.59±0.17 ml/kg/min; P=0.94) and hot (Sg*) (1.32±0.16 vs.1.50±0.13 ml/kg/min; P=0.44) estimates of GE were similar in C and R, with Sg and Sg* denoting glucose effect on both glucose production and disposal and on glucose disposal only, respectively. In conclusion, fasting Gn and the ability of glucose to facilitate its own uptake and suppress its own production is normal in insulin resistant relatives of type 2 diabetic subjects.

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DIETARY CARBOHYDRATE CONTENT MODULATES POSTABSORPTIVE GLUCOSE PRODUCTION BY MODULATION OF GLYCOGENOLYSIS

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Aims: Dietary carbohydrate content modulates postabsorptive glucose production. To investigate whether this effect is attributed to changes in postabsorptive glycogenolysis, we measured postabsorptive glucose production and gluconeogenesis in five healthy males (age 28-55) after 11 days of either high or intermediate-carbohydrate feeding. **Materials and Methods:** Both diets were eucaloric and administered in balanced assignment with an inter-diet interval of 6-8 weeks. The high carbohydrate diet contained 15 % protein, 85 % carbohydrate and 0 % fat (energy equivalents), intermediate carbohydrate diet 15 %, 44 % and 41 %, respectively. Postabsorptive glucose production was measured by infusion of $^2H_2$-glucose, gluconeogenesis by ingestion of ^2H_2O. **Results:** High carbohydrate diet increased postabsorptive glucose production by ~14% (2.34 ± 0.12 vs. 2.06 ± 0.08 mg/kg/min; p<0.05). Although this was associated with a decreased fractional contribution of gluconeogenesis to glucose production of ~12 % (42 ± 1 vs. 48 ± 1 %; $p < 0.05$), the absolute rate of gluconeogenesis did not change (0.99 ± 0.06 vs. 1.00 ± 0.04 mg/kg/min; NS). High carbohydrate feeding increased the absolute rate of glycogenolysis by ~ 38% (1.38 ± 0.09 vs. 1.00 ± 0.04 mg/kg/min; $p < 0.05$). Fasting plasma glucose, insulin, glucagon, cortisol and catecholamines were not different between both diets. **Conclusions:** We conclude that, in healthy males, eucaloric high-carbohydrate feeding increases postabsorptive glucose production by enhanced glycogenolysis and not by modulation of gluconeogenesis.

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EVIDENCE FOR INCREASED GLYCOGENOLYSIS AND GLUCONEOGENESIS IN TYPE 1 DIABETES MELLITUS.

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Aims: Renal glucose release is increased in type 1 diabetes. To determine whether this could be accounted for by increased gluconeogenesis, net balance of major gluconeogenic precursors across the kidney was measured in insulin withdrawn patients with type 1 diabetes before and after insulin infusion.

Materials and Methods: Net renal glucose, lactate, glutamine alanine and glycerol balance was measured in 7 type 1 patients before (B) and after insulin infusion (I) (0.8 mU/kg/min) in order to achieve near-normoglycemia and 10 nondiabetic subjects(ND). Systemic and renal release and uptake of glucose were determined with ^{3}H glucose.Renal plasma flow was determined by the p-aminohippuric acid clearance technique. Overall renal net glucose, lactate and glutamine balance was calculated as RBF x (arterial concentration-renal vein concentration)

Results. Diabetic subjects were markedly hyperglycemic (fasting plasma glucose 15.8±0.9 vs 4.4±0.1 mM in ND,p<0.001) and had increased overall rates of systemic glucose release (1344±98 vs 932±29 µmol/min in ND,p<0.001) Renal glucose release was increased more than two-fold in the diabetic subjects(419±49 vs 204±9 µmol/min in ND,p<0.001). Net renal uptake of major gluconeogenic precursors (lactate 189±20 vs 336±36 µmol/min p<0.01,glycerol 30±4 vs 52±8 µmo/min p<0.05,and glutamine 34±6 vs 48±6 µmol/min NS.) was greater in diabetic subjects, but net renal precursor uptake could account for only 65% of the glucose release in diabetic subjects. Renal net uptake of lactate (I :279±39 vs B:336±36µmol/min p<0.05, glycerol I:14±3vs B:52±8µmol/min p<0.0001 and glutamine I:26±5 vs B: 48±6µmol/min p<0.05) decreased significantly after insulin infusion.

Conclusions. Since renal uptake of gluconeogenic precursors albeit increased, cannot account for the increased glucose release, there may be increased renal glycogenolysis in type 1 diabetes.

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Contribution of Gluconeogenesis to Glucose Production in Mild Type 2 Diabetics.

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Attempts to quantitate gluconeogenesis(GNG) in diabetics have yielded different results. Glucose production is generally accepted to be increased, but recently reported normal or near normal in mild diabetics measured by labeled glucose kinetics with a prime adjusted to plasma glucose concentration. We measured GNG by the new $^2H_20$ method and production (Ra) by infusing (6,6^{2}H)glucose with the adjusted prime in 6 mild type 2 diabetics and 5 controls. Oral antidiabetic agents were withdrawn one week and insulin one day before study. Subjects ingested dinner, 14 kcal/kg body weight at 5-6 PM and then fasted. $^2H_20$ was ingested at 11 PM and 2 AM. (6,6^{2}H)Glucose was infused from 8 AM to 4 PM. Ratio of ^{2}H at carbon 5/2 of blood glucose, equated to % GNG, was determined at intervals between 9 AM and 4 PM. Plasma glucose concentration in controls at 8 AM was 5.2 ± 0.1 mM and declined to 4.9 ± 0.1 at 4 PM. In diabetics 9.6 ± 0.9 mM at 8 AM declining to 7.5 ± 0.9 at 4 PM

	GNG(%)					Ra (µmol/kg/min)	
TIME	9 AM	11 AM	12.30	2 PM	4 PM	11 AM	4 PM
Control	55±3	62±2	62±2	60±1	68±2	9.8±.5	8.6±.5
Diabetes	63±3	69±3	71±3	73±3	74±4	10.6+.5	7.7±.5

Glucose production was no different in diabetics and controls. At each time % GNG was higher, on average 8 %, in the diabetics. In mild type 2 diabetics after an overnight fast there is a small increase in the contribution of GNG to glucose production.

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PARTIAL INHIBITION OF NITRIC OXIDE SYNTHESIS *IN VIVO* DOES NOT INHIBIT BASAL GLUCOSE PRODUCTION IN MAN.

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Basal glucose production decreases linearly by 10-20% in healthy humans for unknown reasons during short-term fasting. This decrease may be explained by paracrine interaction between Kupffercells and hepatocytes. *In vitro*, nitric oxide (NO) inhibits differential pathways of hepatic glucose metabolism but the role of NO on glucose production has not been studied *in vivo* in humans. **Aims**: To study the influence of NO synthesis inhibition with L-NMMA (N^G-Monomethyl-L-arginine) on basal glucose production. **Materials and methods**: 6 healthy men were studied during 14-22 hrs fasting in a saline-controlled crossover study. Glucose production was measured with [6,6-$^2H_2$]-glucose, NO output was measured in exhaled air with a chemiluminescence analyzer, and hemodynamic parameters were assessed using a Finapres model. Data were analyzed using SAS analysis of repeated measurements. **Results**: L-NMMA infusion caused a 40-50% reduction of NO output (t = 0 h to t = 5 h: L-NMMA: 72 ± 8.1 to 36.8 ± 3.9 nl/min/m^2, control: 73.3 ± 6.9 to 72.7 ± 3.6 nl/min/m^2) (p=0.03 L-NMMA vs control) induced a ~22% rise in total peripheral resistance and a ~14% decrease in cardiac output, but did not affect the ~20% decrease in glucose production (L-NMMA: 13.5 ± 0.9 to 11 ± 0.9 µmol/kg/min (p<0.0001), control: 12.8 ± 0.8 to 10.6 ± 0.9 µmol/kg/min (p<0.0001)). Plasma glucose, lactate, alanine and FFA concentrations did not differ between the groups. Glucoregulatory hormone concentrations were the same in both groups except for adrenaline that was lower during L-NMMA infusion. **Conclusions**: This study shows that a 40-50% inhibition of NO synthesis does not influence glucose production *in vivo* in humans, whereas NO influences glucose metabolism *in vitro*. Therefore, NO is not a major direct or indirect modulator of postabsorptive glucose production in healthy subjects.

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ENDOGENOUS GLUCOSE PRODUCTION FROM SMALL INTESTINE IN INSULINOPENIA STATES IN RATS.

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We have recently shown that the gene and activity of glucose-6 phosphatase, the enzyme which catalyzes the release of glucose into the blood, are expressed in the rat small intestine, and are induced by fasting and diabetes. **Aims** : We tested the hypothesis that the increase in the Glc6Pase activity may reflect a gluconeogenic function of the small intestine. **Materials and methods** : Using arteriovenous balance measurements combined with a tracer technique, intestinal glucose uptake (IGU) and release (IGR) have been simultaneously partitionned. Rats were perfused with [3-^{3}H]glucose for 90 min and blood was taken simultaneously in carotide and superior mesenteric vein (after ligation of inferior mesenteric veine) for measurements of concentrations and isotopic enrichments (IE) of glucose. **Results** : After 48 h fasting, the IE of glucose was lower (by 4.6±0.4 %) in mesenteric vs. arterial blood (Mean±SEM, n=8, p<0.001.From this dilution and the intestinal blood flow (6.5±1.6 ml.min^{-1}, cerium microsphere method), the calculated IGR was 8.6±1.4 µmol.kg^{-1}.min^{-1} for a total endogenous glucose production (EGP) of 41.3±1.9 µmol.kg^{-1}.min^{-1} (Mean±SEM, n=8). In postabsorptive streptozotocin diabetic rats, the IE of glucose were also lower (by 1.3±0.4 %) in mesenteric vein than in artery (Mean±SEM, n=12, p<0.01). Taken into account the high blood flow (12.3±1.6 ml.min^{-1}), the IGR was 21.3±7.9 µmol.kg^{-1}.min^{-1}. This IGR being high enough to compensate for the IGU, the glycemia were equilibrated on either side of the intestine (30.3±1.3 vs. 30.6±1.2 mM, artery vs. vein). After peripheral perfusion of [^{14}C]-glutamine or [^{13}C]-glycerol, the IE of glucose were significantly higher in vein than in artery, showing that ^{14}C or ^{13}C from these precursors have been incorporated into glucose synthesized by small intestine. In contrast, alanine and lactate were not precursors of glucose in small intestine. **Conclusion** : These results show that the small intestine contributes to EGP in insulinopenia states in rats, and that the main precursors of glucose are glutamine and glycerol.

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REGULATION OF GLUCONEOGENIC ENZYMES IN SMALL INTESTINE IN FASTED AND DIABETIC RATS.

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We have previously shown that the small intestine contributes to endogenous glucose production in insulinopenia states in rats (companion abstract). The main gluconeogenic substrates utilized by this organ are glutamine and to a lesser extent, glycerol. In contrast, alanine and lactate, the main substrates of gluconeogenesis in the liver, are not utilized in small intestine. **Aims :** To explain these in vivo results, we studied the regulation of expression of the gluconeogenic enzymes, phosphoenolpyruvate carboxykinase (PEPCK) and pyruvate carboxylase (PC), in rat small intestine during fasting and diabetes. **Materials and Methods :** We analyzed PEPCK mRNA by RT-PCR and northern blot, and PEPCK and PC activities in duodenum (DUO), jejunum (JEJ) and ileum (ILE), of fed, streptozotocin-diabetic, and 48h-fasted rats. **Results :** The mRNA and activity of PEPCK were expressed in DUO, JEJ, and ILE in adult rats. The PEPCK mRNA abundance was increased by 30 times in the DUO, by 15 times in the JEJ and by 3 times in the ILE in streptozotocin-diabetic rats. It was normalized upon insulin treatment for 10h. In fasted rats, the mRNA abundance was increased by 17 times in the DUO and JEJ and by 3 times in ILE. It was normalized in both tissues upon refeeding for 7h. PEPCK activity was also increased by 3 to 5 times in diabetic and fasted rats in DUO and JEJ but not in ILE. In contrast, the PC activity was decreased by about 5 times in the JEJ of fasted and diabetic rats whilst it was increased in the liver of the same rats. **Conclusions :** The marked increases in glucose-6 phosphatase (previously reported) and PEPCK gene expression (present study) may account for the induction of gluconeogenesis from glycerol and glutamine in small intestine in insulinopenia states. The dramatic decrease in PC activity in small intestine in the same situation allows us to explain why alanine and lactate are not utilized for small intestine gluconeogenesis, PC being an obligatory step for the conversion of these both subtrates to glucose.

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METABOLISM OF ^{13}C-LABELLED GLYCEROL-1,2,3-TRIS(METHYL-SUCCINATE) IN RAT HEPATOCYTES

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Aims: Glycerol-1,2,3-tris(methylsuccinate) is currently considered as a potential insulinotropic agent in the treatment of NIDDM and nutrient in transplantation procedure. Its metabolism was now investigated in hepatocytes from both control and GK rats. **Methods:** Hepatocytes prepared from overnight fasted normal or GK rats were incubated for 120 min in the presence of 2.5 mM [1,3-^{13}C]glycerol-1,2,3-tris(methylsuccinate) or glycerol-1,2-3-tris(methyl[2,3-^{13}C]succinate). The identification and quantification of ^{13}C-enriched metabolites was achieved by a recently developed method for the deconvolution of NMR spectra with multiplet structures and constraints. **Results:** In control rats, [1,3-^{13}C]glycerol-1,2,3-tris(methylsuccinate) was fully recovered in ^{13}C-labelled glycerol, lactic acid and glucose. The hexose was symmetrically labelled, being mainly enriched with ^{13}C on both C_1 and C_3 and/or C_6 and C_4. In the hepatocytes exposed to glycerol-1,2,3-tris(methyl[2,3-^{13}C]succinate), the recovery of [2,3-^{13}C]succinate, [2,3-^{13}C]fumarate and either double- or single-labelled malate, lactate, alanine and glucose accounted for about half the initial ^{13}C content of the ester. The majority of the glucose molecules were now labelled in both C_1 and C_2 or C_6 and C_5, with a preferential labelling of C_6-C_5 relative to C_1-C_2, the paired C_6/C_1 and C_5/C_2 ratios averaging 1.33 ± 0.04. Comparable results were obtained in GK rats. **Conclusions:** These findings reveal that glycerol-1,2,3-tris(methylsuccinate) is efficiently metabolized in hepatocytes. They reinforce the concept that the asymmetry of glucose ^{13}C-labelling by triose phosphates generated from Krebs cycle intermediates is modulated by the availability of glycerol-derived triose phosphates.

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DIFFERENTIAL EXPRESSION PATTERNS OF THE "LIVER" AND "BRAIN" ISOFORMS OF G-6-P TRANSLOCASE T1.

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Aims: Synthetic derivatives of chlorogenic acid have previously been demonstrated to be potent inhibitors of the hepatic but not islet glucose-6-phoshate translocase T1. The aim of this work was to determine if this apparent selectivity could be explained by the existence of a novel pancreatic isoform of T1. **Materials and Methods:** A Clonetech multiple human tissue blot was probed with a cDNA's representing the 66bp "brain" isoform insert and with a probe which would detect both the "brain" and "liver" isoforms. Additionally T1 was amplified from pancreatic cDNA by PCR using primers described previously for both isoforms. **Results:** Analysis of the Northern blot on a BioRad GS-525 Molecular Imager System reveal that several tissues including heart, liver and skeletal muscle display both the 1.29Kbp and the 1.36kbp isoforms. When probed with the T1 cDNA the pancreas lane displayed 3 major bands and 2 minor bands, however no bands were evident when the same lane was probed with the "brain" specific insert. In addition repeated attempts to PCR the pancreatic T1 from different cDNAs resulted only in bands of 1.29Kbp. When sequenced these bands were all identical to the previously published liver isoform. **Conclusions:** Our results suggest that the predominant isoform of T1 expressed in human pancreas is identical to the 1.29Kbp "liver" isoform. The selectivity of synthetic derivatives of chlorogenic acid for hepatic over islet T1 cannot readily be explained by differential expression.

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MAPPING FRUCTOSE METABOLISM IN THE RAT LIVER

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Aims: Fructose is converted in the liver to glucose and lactate and is also oxidised. We mapped fructose metabolic pathways using a new technique of in situ mapping of hepatic metabolism **Materials and methods:** Livers from 48 hour starved rats were perfused with 1.5 mM lactate. Retrograde digitonin perfusion was used to destroy varying fractions of the lobular volume in different livers, and substrate and metabolite balance and cell pH (pH_i) measured in antegrade perfusions before and during fructose (5 mM) infusion. Plots of residual function or pH_i (^{31}P-NMR) were made against the fraction of remaining viable liver and transformed to provide point-by-point estimates of function along the radius of the lobule. **Results:** Almost all fructose uptake took place in the periportal 50% of the lobular volume. 45% of fructose thus taken up was converted to glucose. Periportal glucose output was doubled during fructose infusion. Both before and during fructose, glucose output fell progressively in a periportal → perivenous direction until it reached zero at 40% along the radius measured from the periportal end. At more perivenous points, uptake of glucose synthesized periportally progressively increased. Periportal lactate uptake ceased 35% and 20% along the radius before and during fructose, when overall uptake was suppressed by 80%. In contrast, at more perivenous sites the lactate output seen before fructose was increased 5-fold by fructose. All metabolite changes during fructose were highly significant ($P<0.001$). There was only a minor fall (0.05 unit) in pH_i in the most periportal cells during fructose, but this fall became progressively ($P<0.02$) more pronounced (up to -0.3 unit) in more perivenous locations, corresponding to the location of increased lactate production. **Conclusions:** Hepatic metabolism of fructose takes place predominantly periportally. Lactate output during fructose infusion is mainly due to perivenous glycolysis of glucose produced from fructose periportally, rather than direct conversion of fructose to lactate.

PS 40
Carbohydrate Metabolism in Muscle

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CREATINE SUPPLEMENTATION AND GLUCOSE METABOLISM

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Aims: Studies *in vitro* show that creatine stimulates insulin secretion and that insulin stimulates muscle creatine uptake. Oral creatine (20g/day) in humans enhances muscle performance and metabolic recovery from exercise by increasing muscle creatine levels and hence substrate for rapid ATP regeneration. Hyperinsulinaemia following chronic creatine ingestion may lead to an insulin resistant state. The aim of this study was to establish the effect on glucose homeostasis and glycogen metabolism of chronic creatine feeding of rats. **Materials and Methods:** 3 groups of 4 rats were fed chow with 0 (CON), 1% or 2% (of diet weight) creatine for 8 weeks (≈10-20g creatine/day in humans). Plasma glucose and insulin, both fasting and after an oral glucose load (3g/kg), muscle creatine and glycogen content and glycogen phosphorylase (GP) and synthase (GS) activities were measured. **Results:** Insulin response to oral glucose was increased in the 2% *cf* the 1% ($p<0.02$, ANOVA) and CON groups ($p<0.03$) while plasma glucose was not different between the groups before or after the oral glucose load.

Table	CON	1%	2%
Fasting insulin (pM)	158 ±15	293±60*	429±75*
Creatine (μmol/g wet wt)	17.6±1.7	22.2±2.1	24.6±0.3*
Glycogen (μmolglu/g wet wt)	16.6±1.4	13.5±0.7	25.2±1.6*
GP activity (U/g wet wt)	56.5±3.6	50.3±9.3	70.1±11.8
GS activity (U/g wet wt)	1.78±0.8	1.93±0.4	1.60±0.3

(Mean±SD); *P<0.01, *cf* CON

Conclusions: This study shows that chronic creatine feeding produces hyperinsulinaemia and creatine accumulation. There were increased muscle glycogen stores despite no change in the activities of GS or GP. Hyperinsulinaemia following oral glucose suggests creatine can cause insulin resistance.

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THE ACTIVITY OF GLYCOGEN SYNTHASE (GS) OF CULTURED HUMAN SKELETAL MUSCLE CELLS IS PROFOUNDLY AFFECTED BY INSULIN.

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Insulin-stimulated glucose uptake is mainly directed into skeletal muscle. Glycogen synthesis is the major pathway of glucose disposal in skeletal muscle, and this is regulated by the insulin-sensitive glycogen synthase (GS). Our aims were to clarify 1) whether cultured human skeletal muscle cells expresses an insulin-sensitive GS and 2) whether insulin affects the allosteric regulation of GS by glucose 6 phosphate (G 6 P), and 3) whether insulin affects the affinity of GS for UDP glucose in these cells. Human satellite cell cultures (HSCC) were established from skeletal muscle biopsies from healthy volunteers. HSCC were, after cell fusion, exposed for 4 days to the following media conditions: A) basal medium (5 mM glucose, 25 pM insulin), B) high-insulin medium (5 mM glucose, 1 μM insulin) and C) high glucose-insulin medium (20 mM glucose, 1 μM insulin). Cell extracts of the cultures grown under conditions A, B, or C were obtained after a 1h exposure to acute basal(I) or high-insulin(+I) media. Preliminary results (n=5, mean +/ SEM) are given in Table. We found that GS in HSCC is insulin-sensitive when precultured in basal medium, and insulin-insensitive when precultured in media containing high insulin concentrations. Acute insulin stimulation increases FV0.1 and decreases A0.5 and Km0.1 in cells precultured in basal medium (A). In cells cultured under hyperinsulinaemic conditions these changes were abolished (B, and C). Thus, GS action is profoundly affected by "long-term" exposure to high insulin concentrations in HSCC. These results demonstrate that the regulation of GS in our cultures of fused human satellite cells is in line with the results from in vivo studies of human skeletal muscle. Thus, our HSCC system seems suitable for studies of the GS regulation in cultures obtained from NIDDM patients.

	$FV_{0.1}$-I	$FV_{0.1}$+I	$A_{0.5}$-I	$A_{0.5}$+I	$Km_{0.1}$-I	$Km_{0.1}$+I
A)	0.13±0.01	0.21±0.03*	0.64±0.08	0.44±0.10"	0.51±0.02	0.37±0.03*
B)	0.16±0.01	0.16±0.02	0.72±0.12	0.72±0.08	0.50±0.02	0.48±0.01
C)	0.13±0.02	0.15±0.02	0.78±0.15	0.67±0.13	0.54±0.15	0.43±0.05

*P<0.05."P<0.1

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IN SAUDI ARABIANS TOO, INSULIN RESISTANCE PREDATES DIABETES

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As Saudi Arabia grapples with the growing problem of NIDDM, virtually nothing is known about the aetiology of the disease in the native population. In particular, whether defects in insulin action and/or secretion predispose to NIDDM remains unexplored. To address this, we measured indices of insulin sensitivity (%S) and β-cell function (%B) using homeostasis model assessment (HOMA) in two groups of young non-diabetic men: one with family history of diabetes (prediabetic, PD, n=60), the other without (control, CT, n=38). The two groups (PD vs. CT) were matched ($p>0.1$) for age (28.6±0.9 vs. 30.0±0.8yrs), BMI (24.6±0.7 vs. 26.0±0.5 $kg.m^{-2}$), and W/H ratio (0.88±0.01 vs. 0.87±0.01). Fasting insulin concentrations were higher in PD group (72±5 vs. 59±5 pM, $p<0.05$). HOMA analysis for %S revealed more than 20% reduction in insulin sensitivity in the PD group (76±5 vs. 94±8, $p<0.05$), implying a significant state of insulin resistance. By contrast, β-cell function was not compromised in PD subjects in whom %B was actually higher than that of CT group (107±4 vs. 97±6, $p=0.04$), thus indicating an adaptive response by β-cell in the face of diminished insulin sensitivity. We therefore, conclude that insulin resistance, not β-cell dysfunction is the predominant precursor of the Arabian brand of NIDDM.

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MUSCLE GLUCOSE METABOLISM DURING ACUTE HYPERGLYCAEMIA AND "NORMOINSULINAEMIA" IN RELATIVES OF TYPE 2 DIABETIC PATIENTS

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Aims: Glucose mediated glucose disposal may be increased in relatives of Type 2 diabetic patients which could be mediated by an increase in glycogen synthase activity by hyperglycaemia. Thus, we investigated intramuscular glucose metabolic pathways during normoinsulinaemic, eu- and hyperglycaemic clamps. **Materials:** Fifteen relatives and 15 age (29.1 vs 32.1 yr), sex and BMI (27.2 vs 26.6 kg/m^2) matched control subjects. **Methods:** Infusion of somatostatin, glucagon, growth hormone and insulin (0.25 mU/kg/min) for 5 hours. Hyperglycemia was induced the last 2 hours. Muscle biopsies were taken during the eu- and hyperglycaemic periods. **Results:** During euglycemia (~6 mM) and "normoinsulinaemia" (~75 pmol/l), intramuscular G-6-P (median (quartiles), 0.66 (0.50-1.26) vs 0.45 (0.31-0.60) mmol/kg dry weight, $p<0.05$) and lactate (4.13 (3.47-5.34) vs 2.99 (2.54-3.88) mmol/l intracellular water, $p<0.01$) concentrations were increased in the relatives compared to the control subjects. However, no differences existed in intramuscular glucose concentration (0.58 (0.47-0.97) vs 0.91 (0.20-1.25) mmol/l intracellular water, NS) or in glycogen synthase activities ($FV_{0.1G\text{-}6\text{-}P}$: 0.37 (0.35-0.41) vs 0.39 (0.31-0.44) %, NS). During hyperglycemia (~12 mM) and unchanged plasma insulin concentration, intramuscular glucose concentrations increased, as compared to euglycaemia, in both relative and control subjects (1.69 (1.21-2.34) vs 1.58 (0.87-2.09) mmol/l intracellular water, NS). However, no difference now existed in the muscle G-6-P concentrations (0.74 (0.52-1.04) vs 0.63 (0.37-0.91) mmol/kg, NS) whereas the small increases in lactate concentrations were still significant (3.94 (3.67-4.78) vs 3.45 (3.03-3.84) mmol/l intracellular water, $p<0.05$). No changes were observed in either groups with regard to glycogen synthase activity at hyperglycaemia (0.36 (0.32-0.42) vs 0.36 (0.29-0.40) %, NS). **Hypothesis:** In relatives of Type 2 diabetic patients, during euglucaemia and low insulin, a post membrane block in glucose metabolism exist with a preferential challenging of glucose into glycolysis (lactate formation). During hyperglycemia, the partitioning of glucose processing into glycolysis and glycogen synthesis is similar in both groups.

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INSULIN-INDEPENDENT MECHANISMS CONTRIBUTE LARGELY TO GLUCOSE DISPOSAL IN MICE

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Aim. Evaluate the relative importance of insulin dependent and insulin independent mechanisms for glucose disappearance. **Methods.** Glucose (1g/kg) was injected iv to anesthetized NMRI mice and 7 samples were taken over 50 min for analysis of insulin and glucose with the minimal model of glucose disappearance. Insulin secretion was the area under the 50 min curve of suprabasal plasma insulin (AUC, min nmol/l), glucose disposal the slope of the glucose disappearance during 20 min (K_G, %/min); insulin dependent glucose disposal was the insulin sensitivity index (S_I) and insulin independent glucose disposal the glucose effectiveness (S_G). The effect of insulin was expressed as the "disposition index" ($DI=AUC \times S_I$). In separate experimental series, circulating insulin was either raised endogenously by giving peptides GLP-1 (10 nmol/kg) or PACAP27 (1.3 nmol/kg), or exogenously by insulin (0.5 or 1 U/kg), or inhibited by diazoxide (1 mmol/kg), all together with glucose. **Results.** K_G was 2.59±0.07 in 190 controls (AUC=17±1), raised to 4.53±0.25 and 4.41±0.33 ($p<0.0001$) by high endogenous (n=51, AUC=68±6) or exogenous (n=52, AUC=41±4) insulin and reduced to 0.49±0.09 ($p<0.0001$) in low insulin condition (n=12, AUC=0.4±0.8). K_G was linearly related to S_G and DI according to $K_G=\alpha S_G+\beta DI$. Parameters α and β were calculated with multiple regression to allow estimation of the relative contribution of S_G versus DI to K_G. S_G contributed to K_G by 65% (controls), 28% (high endogenous insulin), 17% (high exogenous insulin) and ~100% (low insulin), respectively. **Conclusion.** Insulin independent mechanisms account for approximately two thirds of glucose disappearance in normal mice. This large contribution makes it important to establish its molecular mechanism, which may offer new targets for treatment of impaired glucose tolerance.

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METABOLIC RESPONSE TO ANISOOSMOLARITY OF RAT SKELETAL MUSCLE *IN VITRO*.

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Aims: In cultured rat myotubes, exposure to hypoosmotic medium triggers glycogen synthesis, while hyperosmotic medium elicits inhibition of glycogenesis, what led others to hypothesize a role for cell volume changes in the physiologic regulation of muscle glycogen storage. The present study examined the effects of anisoosmotic exposure on isolated rat soleus muscle. **Materials and Methods:** The incubation medium contained different concentrations of sucrose (hypoosmotic, 0 or 65mM; isoosmotic, 130mM; hyperosmotic, 260mM) and 0.05mM glucose, and allowed for an experimental procedure exactly as used in the myotube study. **Results:** As compared to what has been reported for myotubes, native muscle specimens delivered opposite results, because in rat soleus muscle the rates of glycogen synthesis were decreased by hypoosmolarity and increased by hyperosmolarity (nmol glucose into glycogen/g/h: hypoosmotic, 0mM sucrose, 34±3, 65mM sucrose, 33±4; isoosmotic, 65±8; hyperosmotic, 150±11; $p<0.01$ each *vs.* isoosmotic). Further experiments used another medium with a physiologic glucose concentration of 5.5mM, and with the osmotic state varied by mannitol addition (hypo-, iso-, and hyperosmotic at 0, 70, and 120mM mannitol, respectively). Inhibition of glycogenesis by hypoosmotic exposure was also seen (µmol glucose into glycogen/g/h: hypoosmotic, 0mM sucrose, 0.98± 0.19; isoosmotic, 1.72±0.14; $p<0.05$), while hyperosmotic exposure failed to affect glycogen synthesis (hyperosmotic, 1.87±0.23). Furthermore, the observed changes in glycogen synthesis were accompanied by parallel changes in glucose transport and glycolytic flux. **Conclusions:** Our findings indicate that the responses to an anisoosmotic environment observed in native rat soleus muscle are in opposition to what has been described for cultured rat myotubes. The results therefore fail to support the hypothesis that cell swelling is a physiologically relevant stimulator of muscle glycogen synthesis and underline that caution must be applied concluding from myotube cultures to native skeletal muscle.

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NO EFFECT OF LITHIUM ON GLUCOSE HOMEOSTASIS IN OBESE RATS WITH INCREASED MUSCLE GLYCOGEN.

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Aims: Chronic oral treatment with lithium has been shown to improve the metabolic state of partially pancreatectomized insulin-deficient rats in association with a normalization of muscle glycogen content. The present study investigates the effects of lithium in severely insulin resistant obese rats known to exhibit higher than normal tissue glycogen content. **Materials and Methods:** In genetically obese Zucker rats (fa/fa), admixture of 0.3 g/l Li_2CO_3 to drinking water for 3 weeks resulted in an uptake of approximately 8.4 mg/kg/d, what is equivalent to the lower range of therapeutic lithium doses used for the treatment of affective disorders in man. **Results:** In contrast to the insulin-deficient rat model, lithium treatment in obese Zucker rats remained without an effect on basal glycemia (control *vs.* lithium, mg/dl: 136±4 *vs.* 138±10; ns), basal insulinemia (µU/ml: 118±17 *vs.* 110±10; ns), or glucose infusion rate during euglycemic-hyperinsulinemic clamping (M-value, mg/kg/min: 16.3±1.7 *vs.* 16.5±2.0; ns). Although lithium is known to be a potent stimulator of the key enzyme glycogen synthase, glycogen content of various tissues was not affected either (µmol glucosyl units/g: liver, 80.7±5.9 *vs.* 61.6±10.2; gastrocnemius muscle, 20.7±1.7 *vs.* 21.6±0.9; soleus muscle, 12.1±1.2 *vs.* 13.5±1.1; ns each). **Conclusions:** Our results demonstrate failure of lithium to ameliorate derangement of glucose homeostasis in obese Zucker rats. Lithium is therefore effective in partially pancreatectomized rats with decreased tissue glycogen content, but ineffective in obese Zucker rats with increased tissue glycogen content, and hence, the beneficial effect of lithium correlates with prevailing tissue glycogen content. The antidiabetic potential of the glycogen synthase activator lithium in deranged glucose metabolism may therefore depend on the respective state of tissue glycogen stores.

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DIABETIC RAT SKELETAL MUSCLE IS CHARACTERIZED BY DECREASED LACTATE TRANSPORT.

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Impaired lactate metabolism in insulin deficient diabetic rat models as well as in human NIDDM is now well established. On the other hand considerable evidence for a lactate/proton carrier system has been demonstrated in a variety of tissue including skeletal muscle. We hypothetized that diabetic skeletal muscle was characterized by altered lactate exchanges and increased oxidative stress. **Aim.** The aim of the study was to investigate wether streptozotocin (STZ)-induced diabetes resulted in altered lactate exchanges using the rat muscle sarcolemmal vesicles model. **Materials and methods.** Eight Wistar male rats rendered diabetic by streptozotocin injection (65 mg/kg, i.p.) was age-matched to a control group. The initial rate of lactate uptake was measured at various external lactate concentrations in zero-*trans* conditions. Oxidative stress was assessed by measurement of glutathione peroxidase (Gpx) activity and malonedialdehyde (MDA) concentration in red gastrocnemius (RG). **Results.** Fifteen days after diabetes onset, rats had higher blood and muscle lactate concentrations compared with normal rats (2.53 ± 0.17 vs 1.76 ± 0.09 mmol/l and 26.69 ± 1.61 vs 21.86 ± 1.21 mmol/kg w.w for STZ-diabetic and control groups, respectively; $P < 0.05$). STZ-induced diabetes decreased initial rate of total lactate influx at external lactate concentrations from 1 to 100 mmol/l ($P < 0.05$). This decrease in lactate transport was associated with increasing free radical production, as indicated by the increase Gpx activity (84.7 ± 15.01 vs 48.03 ± 3.13 µmol.min^{-1}.mg^{-1} protein) and MDA concentration (100.3 ± 13.5 vs 64.3 ± 8.7 nmol. g^{-1}w.w) in RG. **Conclusions.** We concluded that STZ-induced diabetes decreased total lactate transport activity in rat sarcolemmal vesicles, suggesting that the regulation of lactate transport is not only dependent on muscle activity, but also on lactate levels. We propose that the mechanisms underlying the decreased lactate transport may play a role in muscle insulin resistance.

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Glucose Transporters

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GLUCOSE TRANSPORTER 4 (GLUT-4) EXPRESSION IN HUMAN MUSCLE FIBRES WITH AGE

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Studies of glucose disposal in young and elderly men at various glucose concentrations and at fixed hyperinsulinaemia show decreased maximum glucose uptake rates (vmax) in the older subjects suggesting that increasing age is followed by a decreased glucose-transporter expression. Our aim was to compare the distribution pattern of GLUT-4 in slow vs. fast fibres in sections of skeletal muscle biopsies from healthy fasting volunteers (n=16) at the age of 29 and 64 years, respectively. GLUT-4 immunoreactivity was visualized by an immunoperoxidase reaction, and the fibre type by an immunophosphatase reaction, and the sections were analysed by stereology. Only GLUT-4 immunoreactivity sites associated with the plasma membrane were counted. The fibre type area in each field was estimated by point counting. The expression of GLUT-4 in the two fibre types was estimated by dividing the number of GLUT-4 immunoreactive sites in each fibre type by its area. GLUT 4 expression in slow fibres was 3.21 (2.95 3.70) in young vs. 2.91(2.32 3.23) in older subjects (mean, range, $p<0.04$, Mann Whitney test). In fast fibres, GLUT-4 expression was 2.84(2.62 3.13) in young vs. 2.10(1.89 2.34) in the older subjects (mean, range, $p<0.002$, Mann Whitney test). For both study groups, the GLUT-4 expression in slow fibres was higher when compared to fast fibres ($p<0.007$). Thus, by measuring GLUT-4 immunoreactivity in individual fibres in sections of human skeletal muscle biopsies, we were able to demonstrate that GLUT-4 is more abundantly expressed in slow compared to fast fibres, both in young and elderly individuals. Furthermore, compared with young individuals, older subjects displayed a 25% reduction of GLUT-4 expression in fast fibres, but only a slight decrease in GLUT-4 expression in slow fibres. These results could partly explain both the inter-individual and the age-related variations found in insulin-stimulated glucose uptake in man.

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GLUCOSE TRANSPORTER 1 (GLUT-1) EXPRESSION IN HUMAN SKELETAL MUSCLE IS LOST AT BIRTH

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The presence of the glucose transporter 1 GLUT-1 in human muscle cells has been a matter of debate. Verification of GLUT-1 expression in subcellular fractions of human muscle has been hampered by contamination of erythrocyte membranes, which contain very high levels of GLUT-1. In crude membrane fractions of rat skeletal muscle, it has been shown that about 40% of the GLUT-1 content originates from muscle fibres and the remaining from intramuscular perineurial sheaths (PNS). The aim of this study was to investigate the GLUT-1 immunoreactivity in sections of human skeletal muscle in order to clarify whether human skeletal muscle expresses GLUT-1. Samples of human skeletal muscle were obtained from foetuses of gestational weeks 18, 24, and 40, and from healthy adults. GLUT-1 immunoreactivity was visualized by an enhanced immunocytochemical reaction. Muscle fibres at gestational weeks 18 and 24 all displayed GLUT-1 immunoreactivity. At gestation week 40 and in adult muscle, we only found GLUT-1 immunoreactivity in the sarcolemma in apposition to vessels. While this perivascular staining was general in the gestational week 40 muscle, the staining appeared only sporadically in the adult muscle. In all sections of human muscle, GLUT-1 immunoreactivity was intense in vessels, erythrocytes, and PNS. Our results show that GLUT-1 expression in human muscle is nearly lost at birth and persists only in apposition to vessels at the plasma membrane of muscle fibres.

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ASSOCIATION BETWEEN GLUT4-CONTAINING VESICLES AND PROTEIN PHOSPHATASE-1 IN ISOLATED RAT ADIPOCYTES.

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Aims: The molecular mechanism of insulin-induced glucose transport is unclear. GLUT4 translocation from the intracellular pool to the cell membrane may play an important role in this process. Previous studies have shown that phosphorylation and dephosphorylation of GLUT4 may be involved in insulin-induced GLUT4 translocation, and that protein serine/threonine phosphatase–1 (PP-1) may regulate this process in response to intracellular signal from insulin receptor. However, there is no report regarding the association of PP-1 with GLUT4 and its localiztion in intracellular vesicles. In this study, we investigated whether PP-1 was present in GLUT4-containing intracellular vesicles (GTV), and whether the distribution of PP-1 was altered by insulin in the plasma membrane (PM) and low density microsomes (LDM). **Materials and Methods:** Adipocytes from Sprague-Dawley rats were isolated by collagenase method and incubated with or without insulin. After homogenization, PM and LDM were purified by centrifugation method. GTV was purified from PM and LDM samples by immunoprecipitation using anti-GLUT4 antibody. PP-1 in the GTV samples was detected by Western blot analysis using antibody against PP-1 catalytic subunit. Recombinant PP-1 was used as a positive control. To confirm the presence of GTV, Western blot analysis of these samples was performed using anti-vesicle-associated membrane protein (VAMP) antibody. **Results:** When Western blot analysis of the GTV purified from PM, LDM, and Cytosol was performed using anti-PP-1 antibody; a 39 kDa band was seen at the same level of the electrophoresed positive control. However, insulin did not change the quantity of PP-1 in PM and LDM. Next, to study the localization of PP-1 in GTV, we solubilized the samples with $C_{12}E_8$ during the purification of GTV from PM and LDM by immunoprecipitation using anti-GLUT4 antibody. In the absence of $C_{12}E_8$, VAMP and PP-1 were detected in the immunoadsorbed samples. However, in the presence of $C_{12}E_8$, only PP-1 was detected in the immunoadsorbed samples, VAMP being detected in the supernatants. **Conclusion:** These data suggest that PP-1 is present in GTV, where it is closely associated with GLUT4. Further studies are needed to examine the functional effect of insulin on this GLUT4-associated PP-1.

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HIGH GLUCOSE ALTERS GLUT4 EXPRESSION AND FUNCTION IN PRIMARY CULTURED RAT ADIPOCYTES.

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Aims: Previously it has been shown that chronic exposure to high glucose concentrations in combination with hyperinsulinemia can induce insulin resistance and produce an impairment in both basal and insulin-stimulated glucose transport rates in adipocytes. We studied the impact of glucose and insulin, respectively, on the development of insulin resistance through possible alterations in glucose transport activity and GLUT4 expression in primary cultured rat adipocytes. **Materials and methods:** Isolated adipocytes were obtained by collagenase treatment of epididymal fat from Sprague-Dawley rats. The cells were cultured in DMEM containing 5, 10, 15 or 25 mM D-glucose in the presences or absence of insulin (10^4 μU/ml). After incubation for 24 h the cells were washed in glucose- and insulin-free medium and ^{14}C-D-glucose uptake were assessed during 1h in the presence of different insulin concentrations. Total cellular membranes were assessed by immunoblotting for GLUT4 content. **Results:** Long-term incubation with glucose alone clearly produced a dose-dependent decrease (30-40%) in both basal and insulin-stimulated glucose uptake rates. Surprisingly, GLUT4 content in total cellular membranes was clearly increased (maximally by ~10fold) in a dose-dependent manner as a result of glucose treatment. The addition of insulin during the 24h incubation produced a marked decrease in GLUT4 content, but only when the ambient glucose concentration was high (15 and 25 mM). A parallel reduction (30-40%) in glucose uptake (both basal and acutely insulin-stimulated) was seen following long-term insulin exposure. **Conclusion:** Long-term exposure to high glucose can induce cellular insulin resistance with respect to insulin stimulation of glucose transport, despite an upregulation of GLUT4 content. Thus glucose toxicity may involve an altered functional activity of GLUT4. In contrast, insulin-induced insulin resistance may be explained by an impairment of GLUT4 expression.

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DEXAMETHASONE DECREASES GLUT1 AND GLUT4 CONTENT IN PRIMARY CULTURED RAT ADIPOCYTES

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Aims: Insulin resistance is a well-known effect of glucocorticoid excess. The underlying mechanisms are, however, unknown. The aim of this in vitro study was to investigate the cellular effects of glucocorticoids on glucose transport and glucose transporter proteins 1 and 4 (GLUT1 and GLUT4) in primary cultured rat adipocytes. **Materials and methods:** Rat adipocytes from epididymal fat pads were isolated by collagenase treatment and cultured for 24h with different glucose concentrations (5 and 15 mM) and the glucocorticoid dexamethasone (0.3 μM). After washing, glucose uptake was measured during one hour using ^{14}C-U-D-glucose. GLUT4 and GLUT1 content in total cellular membranes were estimated by immunoblotting. **Results:** The presence of dexamethasone markedly decreased both basal (by 40-55 %, $p<0.01$) and insulin stimulated (by 35-40 %, $p<0.01$) glucose uptake compared to control cells. Total membrane-associated GLUT1 and GLUT4 was decreased in dexamethasone treated cells by 35-60 % compared to control cells. These effects were independent of the glucose concentration in the culture medium. **Conclusions:** These results suggest that dexamethasone decreases both basal and insulin stimulated glucose uptake in rat adipocytes. This may be due to a direct effect of dexamethasone on GLUT1 and GLUT4 expression causing a depletion of the total number of glucose transporters. These alterations may be important in the development of glucocorticoid-induced insulin resistance.

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DIAZOXIDE INCREASES GLUT2 IN THE PLASMA MEMBRANE OF RAT BETA-CELLS.

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Aim: The glucose transporter-2 (GLUT2) is of key importance for glucose stimulation of insulin secretion in normal rat beta-cells. Expression of the Glut2 protein is influenced by glucose but other, not yet identified, mechanisms are also thought to be involved. We examined diazoxide, a potassium channel opener inhibiting insulin secretion, and its short-term effect on Glut2 in isolated rat islets.

Materials and Methods: Islets, isolated by handpicking following collagenase digestion of pancreata of Sprague Dawley rats, were cultured in RPMI medium containing 10% fetal calf serum. The islets were then transferred to serum free media with 5 mM glucose for 1 hour with and without 0.3 mM diazoxide, washed and examined. Separate sets of islets were cultured for another hour at 11 mM glucose before examination. The islets were fixed in Bouin's solution and stained for insulin and Glut2 with specific antibodies and evaluated by confocal microscopy.

Results: Cultures transferred from 5 to 11 mM glucose showed a higher proportion of the Glut2 protein in the cytoplasm. The absolute amount of stainable Glut2 did not differ between the two conditions. The islets exposed to diazoxide had a larger, very predominant, proportion of the Glut2 protein localized to the plasma membrane. This phenomenon was apparent in the cultures exposed to 5 or 11 mM glucose and most pronounced in the 5 mM glucose cultures.

Conclusions: The results suggest that in the rat beta-cell the glucose transporter-2 resides both in the plasma membrane and in the interior of the cell. In short-term experiments, diazoxide causes a shift and increases the amount present in the plasma membrane. Glucose, on the other hand, promotes a cytoplasmic location of the tranporter, a process likely to be ATP dependent.

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EFFECT OF GLP-1 ON GLUCOSE TRANSPORTER GENE EXPRESSION IN DIABETIC STATES

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Aims: GLP-1(7-36) amide (GLP-1) is insulinotropic, has antidiabetic effects, and is insulinomimetic upon glucose metabolism in extrapancreatic tissues. The *in vivo* modulating effect of GLP-1 on glucose transporter proteins in rat liver, skeletal muscle and adipose tissue in streptozotocin-induced non-insulin (STZ-NID) and insulin-dependent (STZ-ID) diabetic models -in which the peptide maintains its insulinomimetic properties-, has been reported. Here, we have studied the action of GLP-1 and insulin, on glucose transporter gene expression in rat liver and skeletal muscle from normal, STZ-NID and STZ-ID rats. Methods: Rats were treated (3 days) -through an osmotic pump- with saline solution, GLP-1 (0.4 nmol/kg/h) or insulin (450 mU/kg/h); Glut-2 and Glut-4 mRNAs were analysed in liver and gastrocnemius muscle, respectively, by Northern blot. Results: liver **Glut-2 mRNA** was augmented in STZ-NID (240±36 % normal saline-treated, n=5, $p<0.05$), and its levels were normalized by GLP-1 treatment (93±32 %, n=6; $p<0.05$ *vs* untreated STZ-NID) and, in a lower extent, by insulin (127±43 %, n=6); no difference in the gene expression between STZ-ID and normal controls was observed, and neither GLP-1 nor insulin treatment modified its value. Muscle **Glut-4 mRNA** was reduced in STZ-NID rats (42±5 % normal saline-treated, n=5, $p<0.001$), and GLP-1 treatment, as insulin, normalized its levels (79±13 %, n=6; $p<0.05$ *vs* untreated STZ-NID); STZ-ID rats showed normal gene expression values which were reduced by GLP-1 (70±17 %, n=6; $p<0.01$ *vs* untreated STZ-ID), but not by insulin. In normal rats, GLP-1 did not alter the glucose transporter gene expression in these tissues. Conclusion: GLP-1 has a role in the control of Glut-2 and Glut-4 mRNAs, either at the transcription level, or over their stability or degradation, in diabetic states.

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DIFFERENT METABOLIC RESPONSE OF ADIPOSE TISSUE AND SKELETAL MUSCLE TO INSULIN IN VIVO

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Aims: Recent data suggest that insulin regulates lipolysis and lactate metabolism in both muscle and adipose tissue. We therefore compared insulin induced antilipolysis and lactate production in human adipose tissue and skeletal muscle using a high range of insulin concentrations.

Material and Methods: The absolute concentrations of glycerol (lipolysis index) and lactate were studied with microdialysis of adipose tissue and skeletal muscle in nine normal-weight volunteers. Each subject was studied twice during euglycaemic, hyperinsulinaemic clamp at 0, 0.15, 0.30, 2.0 and 4.0 mU/kg/min of insulin. The tissue blood flow was measured by the ^{133}Xe washout method.

Results: Baseline glycerol levels in plasma, adipose tissue and muscle were 42.4±6.5, 170.8±10.5 and 83.3±7.9 µmol/l, respectively (p=0.0001, ANOVA). During insulin infusion, glycerol levels were gradually reduced by 47–55% in adipose tisssue and by 35–45% in muscle, the difference between the tissues being significant at each insulin dose (p=0.02–0.004). Basal lactate levels were 0.5±0.0, 1.2±0.1 and 2.7±0.2 mmol/l in plasma, adipose tissue and muscle, respectively (p=0.0001). During insulin infusion, lactate levels increased gradually by 14–294% in adipose tissue and by 10–143% in muscle, the difference between the tissues being significant at the three highest insulin doses (P=0.05–0.0009). In neither tissue did the tissue blood flow change during the experiment.

Conclusions: In normal subjects, adipose tissue is much more reactive to insulin than skeletal muscle concerning inhibition of lipolysis and stimulation of lactate production indicating that adipose tissue is a major target for insulin action on lipid as well as glucose metabolism.

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INSULIN SIGNALLING TO METABOLIC EVENTS IS IMPAIRED IN SKELETAL MUSCLE FROM PEOPLE WITH NIDDM

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Aims: Skeletal muscle insulin resistance is a characteristic feature of NIDDM. The aim of this study was to characterise insulin signalling pathways in skeletal muscle from NIDDM subjects. **Methods:** 12 NIDDM and 17 control subjects participated in the study. Subjects were matched for age (57.3±1.8 *vs.* 56.1±1.1), BMI (27.5±1.0 *vs.* 25.9±0.5), percent body fat (23.4±2.0 *vs.*24.3±1.2) and physical fitness (29.5±1.5 *vs.*31.5±1.3) for NIDDM *vs.* controls respectively. Whole body glucose utilisation, assessed by the hyperinsulinaemic-euglycaemic clamp procedure, was markedly decreased in NIDDM compared to controls (M value equal to 27.8±4.0 *vs.* 40.6±3.3 mmol glucose/kg/minute, p=0.02). Insulin-signalling events were determined in isolated skeletal muscle from NIDDM and control subjects incubated in the presence of 0.6, 2.4, or 120 nM insulin. **Results:** Despite marked whole-body insulin resistance, insulin-induced tyrosine phosphorylation of the insulin receptor β-subunit was similar between NIDDM and controls. Furthermore, insulin-stimulated glycogen synthase activity and phosphorylation of Map kinase and was similar between the groups. In contrast, IRS-1 tyrosine phosphorylation was markedly reduced in NIDDM subjects, especially at high insulin concentrations (40% of control levels at 120 nM, p=0.05). Furthermore, antiphospho-tyrosine associated PI3 kinase was also impaired in skeletal muscle from NIDDM compared to controls, achieving 51% and 55% of control activity at 2.4 and 120 nM insulin (P=0.1 and 0.003 respectively). These post receptor defects were associated with impaired insulin-stimulated glucose transport in NIDDM muscle at all insulin levels tested. Importantly, protein expression of the insulin receptor, IRS1, IRS2, Map kinase, or glycogen synthase were similar between NIDDM and controls. **Conclusions:** Insulin signalling pathways are differentially impaired in skeletal muscle from lean NIDDM subjects, such that IRS1 phosphorylation, PI3 kinase activity and glucose transport are severely impaired, while insulin receptor tyrosine phosphorylation and further signal transduction to Map kinase and activation of glycogen synthase is unaffected. Thus, impaired insulin-signal transduction in skeletal muscle from lean NIDDM subjects appears to be related to functional defect(s) rather than a result of aberrant protein expression.

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MUSCLE GLUCOSE UPTAKE IS EFFECTIVELY ACTIVATED BY ISCHEMIA IN NIDDM SUBJECTS.

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Aims: To evaluate the function of the Wortmannin insensitive pathway for activation of muscle glucose uptake in NIDDM. **Materials and methods**: The ability of ischemia to stimulate muscle glucose uptake was investigated in 9 NIDDM patients and 9 healthy control subjects (f-glucose; 9.4±0.8 vs. 5.1±0.1 mmol/L, p<0.001 and f-insulin; 8.1±2.6 vs. 4.5±0.7mU/L, p<0.05 NIDDM and controls respectively), matched for sex, age and BMI. Arterial plasma and interstitial (measured by subcutaneous and muscle microdialysis) concentrations of glucose and lactate were recorded in fore-arm at basal and during ischemia and post-ischemia. **Results:** During ischemia, muscle interstitital glucose concentration decreased significantly from 7.7±0.6 to 5.4±0.4 (p<0.01) and from 4.4±0.3 to 3.6±0.3 mmol/L (p<0.05) in NIDDM and controls respectively. The arterial-interstitial (A-I) glucose difference was 1.7±0.6 and 0.7±0.3 mmol/L at basal and increased significantly to 3.5±0.7 (p<0.01) and 1.4±0.3 mmol/L (p<0.05) during ischemia in each group, respectively. Interstitial lactate raised significantly during ischemia in both groups, from 0.8±0.1 to 1.1±0.1 (p<0.05) and from 0.5±0.1 to 0.9±0.2 mmol/L (p<0.05) respectively. A-I glucose concentration difference was abolished in post ischemia and regained after ~ 15 min whereas high interstitial lactate levels remained elevated throughout the study. Subcutaneous interstitial glucose concentration remained unchanged during ischemia and post-ischemia in both groups, whereas interstitial lactate concentration increased during ischemia from 1.4±0.2 to 2.0±0.2 (p<0.05) and from 1.1±0.1 to 1.8±0.3 mmol/L (p<0.05) in NIDDM and controls, respectively. Plasma glucose and lactate were unchanged in both groups during the study period. **Conclusions:** The results show that muscle but not fat cell glucose uptake is readily activated by ischemia in insulin resistant NIDDM subjects, suggesting the effective activation of a pathway alternative to that of the insulin signal in muscle cells.

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LOCAL INFLUENCE OF INSULIN INFUSION ON TISSUE GLUCOSE CONCENTRATION

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Tissue glucose (TG) monitoring could become in the near future a diagnostic tool to regulate the insulin delivery in diabetic patients. However, insulin may locally influence the TG concentration and, when infused at the site where glucose is monitored, this may lead to errors in the TG monitoring. **Aim:** We designed a study to investigate the local influence of insulin infusion on TG concentration. **Methods:** A total of 15 experiments were performed in healthy volunteers (8w:7m; age: 24.9±3.3 years; BMI: 23.5±3.1 kg/m²). Three microdialysis probes were inserted in the abdominal s.c. tissue. All 3 probes were perfused with phosphate buffered saline at a constant flow rate (0.3 µl/min) throughout the whole experiment (t = 6h). Dialysates were collected on 30 min fractions. Two infusion catheters were implanted next to 2 microdialysis probes (distance between probes and catheter: 0.7±0.3 cm, distance measured by ultrasound). One catheter was used to infuse human regular insulin 1 IU/h and a 3 IU bolus, while the other was used to infuse NaCl 0.9% saline (same volume as insulin volume was infused). The glucose concentration of the dialysates was measured at the end of each experiment **Results:** No statistically significant difference (p=0.18) was obtained between the correlation of the glucose concentration influenced by the saline infusion and the control (r=0.91±0.09) and the correlation of the glucose concentration influenced by insulin and the control (r=0.87±0.14). **Conclusions:** According to these data we may conclude that insulin infusion does not locally affect the TG concentration.

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INTERDEPENDENCE OF MECHANISMS OF INSULIN-INDUCED CORONARY VESSELS DILATATION

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We have previously shown that insulin induce dilatation of coronary arteries (CA) in dogs and that the effect is independent from changes in heart function and myocardial metabolism. Aim of this study was to examine interdependence of possible mechanisms of insulin action on CA in vivo. Materials and Methods: Experiments were performed on 25 dogs under chloralose anaesthesia (40-100mg/kg, i.v.). Catheterization and extracorporal programmed autoperfusion of left CA, catheterization of heart and continuous drainage of coronary sinus (CS) were used. Myocardial uptake (MU) of substrates were determined by calculation of coronary arteriovenous difference (CAVD). Results: I.v. injection or intracoronary infusion of insulin (0.1-1.0 IU/kg or 0.01 IU/min, resp.) caused dose-dependent stepwise dilatation of CA. At the nadir of hypoglycaemia on 30th min after injection of high dose of insulin (1.0 IU/kg) CA perfusion pressure, left ventricular dP/dt and arterial blood pressure decreased (by 12.0 ± 1.0%, 15.5± 3.3% and 11.6 ± 3.1%, resp.) and CS blood O_2 saturation increased (by 15.5 ± 4.3%). CAVD by glucose increased from 0.41 ± 0.05 to 0.69 ± 0.08 mmol/l and by NEFA decreased from 0.20 ± 0.06 to 0.08 ± 0.02 mmol/l. Changes of lactate and pyruvate MU were unsignificant. Correction of hypoglycaemia by i.v. glucose infusion did not abolish insulin-induced CA dilatation. Insulin-induced CA vasodilatation was inhibited by CA infusion of NOS blocker Nω-nitro-L-arginine methyl ester (1.0 mg/min), or by guanilyl cyclase blocker methylene blue (5 mg/min), or intracoronary injection of Na+,K+ATPase blocker ouabaine (25-100 μg) or i.v. injection of atropine (0.5 mg/kg) independently.Conclusions: We suggest that insulin makes use of all above mentioned mechanisms as essential steps in the vasodilatatory signal transduction. However a possibility remains that the different mechanisms use common part of vasodilatatory reserve.

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ACUTE INSULIN ADMINISTRATION INCREASES LDL CHOLESTEROL OXIDATIVE STRESS IN VIVO

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Aim: Type 2 diabetes, essential hypertension and dyslipidaemia are conditions of enhanced oxidative stress and atherosclerosis. We tested whether any relationship exists between insulin resistance and oxidative stress in humans. Materials and Methods: In 23 healthy volunteers, we measured vitamin E content of LDL-cholesterol (LDL-CH), vulnerability to oxidation of the circulating LDL-CH particle (measured as the lag-phase of *in vitro* copper-sulphate-induced oxidation), and malondialdehyde (MDA) production following *in vitro* challenge of LDL with human umbilical vein endothelial cells (HUVEC), at baseline, after two hours of saline infusion, and after two hours of euglycaemic hyperinsulinaemia (7 $pmol \cdot min^{-1} \cdot kg^{-1}$ insulin clamp). Results: Vitamin E contents of LDL-CH were 6.78±0.06 and 6.77±0.06 mg/mg at baseline and after saline, respectively. After 2 hours of euglycaemic (5 mM) hyperinsulinaemia (400 pM), vitamin E in LDL-CH decreased to 6.64±0.06 mg/mg (-4%, $p<0.04$). LDL-CH oxidative lag-phase decreased from basal values of 108±3 and 107±3 min (baseline and saline) to 101±3 min following insulin (-7%, $p<0.0001$). Cell-mediated MDA concentrations were 4.96±0.11 and 4.98±0.10 nM at baseline and following saline, respectively; and rose to 5.28±0.10 (+6%, $p=0.0006$) following insulin. The insulin-induced reduction in oxidative lag-phase was directly related to the insulin-induced decrement of vitamin E content of LDL ($r=0.55$, $p<0.01$). At baseline, LDL-CH oxidative lag-phase was directly related to both systolic and diastolic blood pressure values ($r=0.42$,$p<0.05$ for both). The shortening of lag-phase after insulin infusion was higher in patients with higher baseline serum triglyceride concentrations. None of the in vitro oxidation parameters was related to insulin sensitivity, as determined by the clamp. Conclusion: Acute, physiological hyperinsulinaemia depletes LDL vitamin E content, and enhances both copper-induced and cell mediated LDL-CH oxidation *in vitro* independently of insulin sensitivity. Thus, insulin per se may be a pro-oxidant *in vivo* in humans.

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ACUTE EFFECTS OF INSULIN ON SERUM SOLUBLE ADHESION MOLECULES AND PLASMA HOMOCYSTEINE LEVELS

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Aims: Hyperinsulinemia is well known to induce the atherosclerosis. Adhesion molecules on endothelial cells play an important role in the development of atherosclerosis, and serum levels of soluble type of adhesion molecules increase in patients with atherosclerosis. Hyperhomocysteinemia has been also recognized as one of coronary risk factors. But there are few reports on the relationship between hyperinsulinemia and serum soluble adhesion molecules or plasma homocysteine levels. Thus, we investigated whether acute hyperinsulinemia changes serum soluble adhesion molecules or plasma homocysteine levels. **Methods:** We measured serum levels of sVCAM-1, sICAM-1 and sE-selectin and plasma homocysteine levels in fasting and during a hyperinsulinemic euglycemic clamp in nine healthy men (26.7±3.1(mean±SD) years; BMI, 22.4±2.2 kg/m^2) without hypertension, glucose intolerance, or hyperlipidemia. The clamp study was performed as follows: each subject was connected with the artificial pancreas (Nikkiso STG-22) and received a constant infusion of insulin (Novolin R) for two successive 90-minute period at rates of 0.5 and 3.0 mU/kg/min, respectively. Serum levels of soluble adhesion molecules were measured by ELISA, and plasma homocysteine levels by HPLC. **Results:** During the clamp study, serum insulin levels increased from 38.4±24.0 pmol/l at baseline to 234.6±75.6 pmol/l and 1464.0±214.2 pmol/l at 90 min and 180 min, respectively. Plasma homocysteine levels decreased 11.9±1.5 nmol/ml to 10.3±1.4 nmol/ml ($p<0.05$) and 9.5±1.4 nmol/ml ($p<0.01$), respectively. However, serum levels of soluble adhesion molecules were essentially unchanged. **Conclusions:** Acute hyperinsulinemia induced the reduction of plasma homocysteine levels, but did not influence the levels of circulating adhesion molecules in healthy men. The significance of this phenomenon remains unclear. Further investigation will be needed to clarify the effects of acute hyperinsulinemia on plasma levels of homocysteine and circulating adhesion molecules in patients with multiple metabolic abnormalities.

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INSULIN AND LEPTIN ACTIVATE STAT6 IN FAO HEPATOMA CELL LINE

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Aims: Upon insulin binding the insulin receptor triggers a cascade of phosphorylation events that lead to changes in the expression of specific genes. Leptin belongs to the family of cytokine receptors, acting mainly through the JAK/ Stat signaling pathway. There are increasing number of evidence showing crosstalk between insulin and cytokine pathways. To investigate the possible interactions between the insulin and JAK/ Stat pathway we evaluated insulin action on Stat proteins in Fao hepatoma cells, a well differentiated, highly insulin responsive cell line that express both the short and long forms of leptin receptor. **Materials and methods:** Fao rat hepatoma cells were starved and stimulated with 100nM insulin or 60nM leptin. Tyrosine phosphorylated Stat proteins were detected by immunoprecipitation and immunoblotting with antiphosphotyrosine antibody. Nuclear translocation was measured by detecting Stat6 in the cytoplasmic and nuclear lysates. DNA binding capacity was measured by gel shift assay. **Results:** Insulin induced tyrosine phosphorylation and concomitant nuclear translocation of Stat6 (IL-4Stat) at 5 to 10 minutes of incubation. The nuclear Stat6 content increased by 150-200% and persisted till 30 minutes of stimulation. This effect was also accompanied by a rapid 10-15fold increase in the DNA binding ability of Stat6, peaking at 10minutes and rapidly decreasing to basal level by 30 minutes. Insulin had no effect on other Stat proteins. Leptin also caused comparable nuclear translocation of Stat6 while had no detectable effect on Stat3. The kinase(s) involved in Stat6 activation are yet to be clarified but preliminary data suggest JAK2 to play a role in it. **Conclusion:** In summary our observations suggest a common nuclear effector molecule, Stat6, for insulin and leptin in liver which could play a role in alterations in gene expressions and metabolic processes observed in obesity and other insulin resistant states.

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INTERACTIONS OF α-LIPOIC ACID AND γ-LINOLENIC ACID ON INSULIN ACTION IN INSULIN-RESISTANT RAT MUSCLE.

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Aims: We assessed the individual and interactive effects of α-lipoic acid (ALA, an antioxidant) and the n-6 essential fatty acid γ-linolenic acid (GLA, a prostaglandin precursor) on insulin action in the insulin-resistant obese Zucker rat. **Materials and Methods:** ALA, GLA, and a unique conjugate consisting of equimolar parts of ALA and GLA (ALA-GLA) were given daily for 14 days at 30 mg/kg body weight. **Results:** Plasma insulin was reduced 21% ($p<0.05$) by ALA, 7% (non-significant (NS)) by GLA, and 28% by ALA-GLA. Plasma free fatty acids were reduced 18% by ALA, only 4% (NS) by GLA, and again to the greatest degree (27%) by ALA-GLA. Individually, ALA and GLA both caused significant improvements (decreases of 23% and 25%) in the glucose-insulin index (product of glucose and insulin areas under the curve during an oral glucose tolerance test, and an indirect index of peripheral insulin action). ALA-GLA elicited the greatest reduction in the glucose-insulin index (38%). These improvements in whole body insulin action were likely mediated by enhanced skeletal muscle glucose transport, as insulin-stimulated (2 mU/ml) 2-deoxyglucose uptake in the isolated epitrochlearis and soleus muscles were increased by 31-41%, 20-35%, and 57-63% in the ALA, GLA, and ALA-GLA groups, respectively. **Conclusions:** These results indicate that the unique conjugate of the antioxidant ALA and the n-6 essential fatty acid GLA elicits significant improvements in whole body and skeletal muscle insulin action on glucose disposal in the insulin-resistant obese Zucker rat. The action of this ALA-GLA conjugate appears to be due to the additive effects of its individual components on these variables.

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NOVEL DNA REGULATORY REGIONS FOR SYNERGISTIC INDUCTION OF FATTY ACID SYNTHASE GENE BY INSULIN AND GLUCOCORTICOIDS

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Aims: Dyslipidaemia associated with insulin resistance might involve also a defect in fatty acid biosynthesis. The fatty acid synthase (FAS) is regulated at the transcriptional level by nutrients and hormones. It has been found that insulin and glucocorticoids induce synergistically FAS gene transcription. However, the DNA regulatory sequences have not been identified as yet. The objective of the study was to examine the 5'-flanking region (up to -16000 bp) of the FAS gene for new DNA regulatory sequences responsible for synergistic response of the gene to insulin and glucocorticoids. **Methods:** The regulatory sequences were first identified in rat liver and spleen using the *in vivo* DNase I hypersensitivity approach, and than *in vitro* functionally characterized using primary hepatocytes transfected with different DNA fragments (from -9700 to +65 bp) linked to the chloramphenicol acetyltransferase reporter vector. **Results:** 48 h of fasting followed by refeeding of rats were used as physiological stimuli to change plasma insulin and glucocorticoids levels. Two novel hypersenstivity (HSS) sites have been detected between -8650 and -8550 bp (HSS 1) and between - 7200 and -7100 bp (HSS 2) in rat liver but not in spleen. 6 h refeeding of fasted animals increased the intensity of HSS 2 by twofold (48 h fasting: 9.4±2.5 A.U.; 48 h fasting + 6 h refeeding: 21.2±4.8 A.U. /arbitrary units/; $p<0.01$). On the other hand, the intensity of HSS 1 site did not change. The *in vitro* functional characterization localized a synergistic response to insulin and dexamethasone in the -7382/-4605 bp fragment ligated to the proximal promotor (I+D: 12.7±3.2; I: 4.6±0.7; D: 3.5±0.7; fmol/min/μg; $p<0.01$). Deletion of HSS 2 region from the fragment yielded a loss of the synergistic induction of the FAS gene. However, the HSS 2 alone (the DNA fragment -7382/-6970 bp) linked to the promotor was not sufficient for the full induction of FAS gene. **Conclusion:** a) two new tissue specific DNA regulatory regions have been identified on the FAS gene; b) for synergistic induction of FAS gene transcription by insulin and glucocorticoids the distal regulatory sequences between -7382 and -4605 bp are required c) which has been confirmed under both *in vivo* and *in vitro* conditions.

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INHIBITION OF PERIPHERAL INSULIN REMOVAL IMPROVES SENSITIVITY TO INSULIN: DOSE RESPONSE STUDIES WITH METFORMIN.

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Aims: Metformin has been suggested to have effects on peripheral insulin action. The mechanisms are not yet clear. These studies were therefore designed to assess the interaction between metformin and insulin in peripheral tissues by perfusing rat hindquarters with a sub-clinical insulin dose and metformin at several concentrations. **Materials and Methods:** Hindquarters from 24h-fasted rats were perfused (recirculating, 11.5ml/min) for 120min with oxygenated human erythrocytes in buffer, containing 5.6mmol/L glucose. The following groups (n=5 each) were studied: (i) control (no additions) (ii) insulin alone infused at a rate to reach ~150pmol/L (iii) insulin at the same infusion rate as in (ii) + 2ug/ml metformin, (iv) + 10ug/ml metformin and (v) + 90ug/ml metformin in the perfusate medium. Glucose was infused at variable rates to maintain basal glycemia. Glucose uptake and insulin extraction by the hindquarter were determined from differences of glucose and insulin concentrations between inflows and outflows. **Results:** Total glucose uptake was (i) 46±3mg (ii) 50±1mg (iii) 63±2mg* (iv) 74±1mg* and (v) 80±1mg* (*, $p<0.05$ vs control) and insulin concentrations increased in parallel: (i) 0 (ii) 17±1 (iii) 20±2 (iv) 22±1 and (v) 27±2 (shown as insulin area nmo/l-120min) ($r=0.7$, $p<0.05$), secondary to a decreased insulin extraction: (ii) 8.6±0.9% (iii) 6.7±0.9% (iv) 6.5±0.1%* (v) 4.8±0.4% ($p<0.05$ vs (ii)). Metformin alone, at low doses, did not have any effect on hindquarter glucose uptake. **Conclusions:** These data indicate that metformin at therapeutic concentrations can influence peripheral insulin action by reducing insulin removal by muscle. Since glucose uptake increases, it is suggested that the decreased insulin extraction causes a rise in its interstitial levels. Alterations in peripheral insulin extraction, although not necessarily systemically detectable *in vivo*, may therefore contribute to the local determination of insulin action, and thus to systemic insulin sensitivity.

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MECHANISM OF ATTENUATION OF BASAL ENDOGENOUS GLUCOSE PRODUCTION BY METFORMIN IN HIGH-FAT FED RATS.

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Aims : To understand the molecular mechanism of action of metformin on endogenous glucose production (EGP) in insulin resistance states, we assessed EGP (basal and insulin clamp) and liver metabolites and enzymes in rats fed a high-fat diet for 6 weeks, supplemented (HFM) or not (HF) with metformin at a human therapeutic dose (50 mg/kg/day) for the last week. **Results** : Basal EGP (determined by [3-^{3}H]glucose dilution after 3 h of saline perfusion in postabsorptive anesthetized rats) was lower in HFM than in HF rats (41±0.8 vs 52±2 μmo/kg/min, means±SEM, n=5, $p<0.01$). Plasma insulin was high (refering to normal rats under similar conditions) but not different (234±24 vs 278±58 pmol/L) in HFM vs HF rats. Plasma glucagon, glucose, TG and FFA were not different in both groups. The hepatic contents in glucose-6 phosphate (118±12 vs 38±8 nmol/g wet liver, $p<0.01$) and in glycogen (19.6±4 vs 3.8±2.5 mg/g, $p<0.01$) were higher in HFM than in HF rats. This was not due to glycogen synthase a, glycogen phosphorylase a, or gluco-kinase activities, which were not different in both groups. This might be due to the attenuation of glucose-6 phosphatase (Glc6Pase) activity in HFM rats with regards to HF rats (7.9±0.4 vs 10.3±0.9 μmol/min/g liver, $p<0.05$). The latter was likely due to a posttranslational effect since the amount of Glc6Pase protein (western blot) was not different in both groups. Upon insulin perfusion (480 pmol/h) with maintenance of glycemia, EGP was decreased (weakly, refering to normal rats under the same conditions) to the same levels in both groups (29±9 and 31±2 μmol/kg/min in HFM and HF rats, respectively, $p<0.05$ and 0.01 vs respective saline perfused controls. **Conclusion**: These data strongly suggest that : 1) a major action of metformin in insulin resistant HF rats involves a decrease in EGP, which might be dependent on an attenuation in the liver Glc6Pase activity ; 2) metformin action is allowed to take place at basal rather than at elevated insulin levels.

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MUSCLE PROTEIN KINASE B PHOSPHORYLATION IS RESTORED WHEN FAT-INDUCED INSULIN RESISTANCE IS REVERSED

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Chronic high-fat feeding in rats causes insulin resistance, impairing insulin-stimulated glucose uptake in skeletal muscle. However, this insulin resistance can be readily reversed by a single, high-glucose, low-fat meal. Protein kinase B (PKB) is phosphorylated and activated by PI3-kinase in response to insulin and has a putative role in glucose transport and glycogen synthesis. **Aim:** To compare the degree of muscle PKB phosphorylation in control chow fed (C), high-fat fed (HF-F) and high-fat fed rats given a single high-glucose meal (HF-G). **Materials and Methods:** Immunoreactive PKB phosphorylation (Ser473) was determined under basal and euglycaemic-hyperinsulinaemic clamp (0.25U/kg/h) conditions, and 3min after a 1U iv bolus of insulin. **Results**: High-fat feeding significantly reduced muscle glucose uptake (Rg'), but this was restored after a single high-glucose meal. Basal PKB phosphorylation was unaltered with high-fat feeding but was increased after a single high-glucose meal. Insulin-stimulated clamp PKB phosphorylation was decreased by 33% in HF-F rats and restored in HF-G. PKB phosphorylation was stimulated 100-fold over basal in rats given insulin intravenously, but no difference was observed among groups.

	Muscle Rg'	PKB Phosphorylation (O.D. units)		
	$\mu mol.100\ g^{-1}.min^{-1}$	Basal	Clamp	1U Insulin
C	20.1±2.5	2.0±0.9	13.6±1.2	280±70
HF-F	12.9±1.0**	3.1±0.5	7.9±0.8*	320±30
HF-G	25.4±3.2††	5.3±1.2*	13.7±1.8†	290±50

means ± SE (n=4-6). ANOVA *p<0.05, **p<0.01 vs C, †p<0.05, ††p<0.01 vs HF-F

Conclusions: Although the responsiveness of muscle PKB phosphorylation to a maximal insulin bolus is unaltered, under physiological (100mU/l) clamp insulin conditions PKB phosphorylation is reduced in insulin resistant high fat fed rats. Both clamp insulin-mediated muscle glucose uptake and PKB phosphorylation can be restored by a single overnight high glucose, low fat meal. These data support the importance of PKB phosphorylation in *in vivo* insulin signalling.

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THE INFLUENCE OF DIETS RICH IN SATURATED FATTY ACIDS DURING AND AFTER GESTATION ON INSULIN SENSITIVITY IN UNANAESTHETIZED RATS

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Diets rich in saturated fatty acids induces insulin resistance. Restriction of protein or energy intake during gestation or early life has also been linked to developmental defects in the endocrine pancreas as well as insulin resistance. It is not known whether saturated fatty acids given to mothers during the gestational period may be detrimental to glucose metabolism of the offspring. The question raised is important in the light of the massive consumption of saturated fat in the developed countries. An animal study was designed to answer this question. Female Wistar rats were given isocaloric diets rich in carbohydrate (C, 80E%) or fat (F, 58E%, mainly saturated fatty acids) during gestation. The F-generation was split into five subgroups: 1 and 2 continued on diet C or F in the suckling and weaning period until week 16. Group 3 with mothers on diet C continued on the C diet during the suckling period but changed to a F diet at weaning until week 16. Group 4 with mothers on diet F continued on diet F during the suckling period but changed to diet F at weaning until week 16. For group 5 the offspring of mothers given a F diet was changed to C diet during suckling until week 16. Hyperinsulinemic euglycemic clamps were performed in conscious, unrestrained rats to determine the insulin sensitivity (n=9-12 per group). The steady state p-glucose levels during the clamps were not different between groups, however the steady state insulin levels were higher in group 3 ($p<0.05$) and tended to be higher in group 2 as well. The glucose infusion rate (mg/kg/min) was lowered to the same extent in both groups receiving diet F (group 2 and 3) (24.7±2.0 and 22.0±1.9, respectively) whereas the other groups were similar (1: 32.2±2.3, 4: 27.9±1.7 and 5: 26.9±1.6). The rate of glucose disappearance (using ^{14}C-glucose) (Rd: mg/kg/min) were in group 1: 30.4±3.0, 2: 25.1±1.6, 3: 24.0±1.8, 4: 29.5±2.7 and 5: 25.2±1.5. No significant differences in the calculated hepatic glucose output was detected. In conclusion, dietary fatty acids given during gestation and/or the suckling period does not seem to have deleterious effects on the insulin sensitivity later in life. The well known effect of dietary saturated fatty acids on lowering of whole body insulin sensitivity is confirmed.

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FEEDING INCREASES INSULIN ACTION BY A HEPATIC PARASYMPATHETIC NEUROHUMORAL MECHANISM

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AIM: The response to insulin has been proposed to depend upon a hepatic parasympathetic nerve (HPN) reflex release of a hormone that sensitizes skeletal muscle to insulin. Hepatic denervation or atropine results in insulin resistance. In previous studies, rats were fed ad-lib but the duration since feeding was unknown. We tested the hypothesis that rats in the postprandial period have a HPN-dependent component of insulin action and that fasting reduces this component and results in reduced insulin action. **METHODS**: The fasted group was tested without refeeding; the fed group was given food for 1 hr. and tested immediately thereafter or for specific times after the feeding period (6, 12 and 18 hr.). Insulin sensitivity was tested using the rapid insulin sensitivity test (RIST) with the RIST index being the amount of glucose infused over 30 min following 50 mU/kg insulin using a euglycemic clamp. **RESULTS**: The fed rats had a control RIST index of 224±18 mg/kg which was reduced to 77±19 after atropine (1mg/kg, i.v.). Control RIST index decreased with duration of fast ($p<0.005$). The post-atropine index was similar in all groups. HPN-dependent insulin action decreased with duration of fast ($p<0.005$). **CONCLUSION**: Fasting leads to reduced insulin sensitivity. Feeding leads to increased sensitivity dependent on the time since feeding with the increase due to the HPN-dependent component of insulin action.

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BETA-CELL MASS AND PORTAL VENOUS DRAINAGE OF THE PANCREAS ARE INDEPENDENT DETERMINANTS OF GLUCOSE TOLERANCE.

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Aims: Either peripheral entry of insulin or a decrease in B-cell mass alone, can decrease glucose tolerance. On the other hand, if hyperinsulinemia secondary to bypassing the first pass uptake of glucose by the liver, induces insulin insensitivity, then hemipancreatectomy (H) could ameliorate the insensitivity following diversion. The interaction of H and the peripheral diversion of pancreatic veins on systemic and hepatic insulin sensitivity in the context of an intravenous glucose infusion was therefore studied. **Materials and Methods:** Four groups of dogs were used: (i) D - pancreatic venous drainage diverted from the portal vein to the inferior vena cava (n=6) (ii) S - sham operation with reanastomosis of the veins to the portal vein.(n=6) (iii) H - with resection of the tail of the pancreas (n=7) (iv) HD - both resection and diversion (n=11). Following recovery from surgery, each animal was fasted for 18h and underwent an infusion of glucose at 10mg/kg-min. A concurrent infusion of [^{14}C-1]glucose was used to measure the glucose output rate (HGO) and its metabolic clearance rate (MCR_g).Insulin sensitivity S_I was quantitated as the ratio of MCR_g to circulating insulin. **Results:** An improvement in glucose tolerance in D and deterioration in H was demonstrated, with mean glycemia of 134±6 157±7, 190±17 and 174±7mg/dl in D,S,H and HD respectively. Mean insulin levels were the same in S and H (32±9 and 30±8 uU/ml) and in D and HD (55±7 and 50±6 uU/ml). With very similar MCR_g (~7ml/kg-min), S_I fell from 0.38±0.13 and 0.32±0.08 for S and H to 0.17±0.03 for both D and HD. HGO was suppressed by 71±8% and 71±13% for D and S and by only 44±14% and 47±6% for H and HD. **Conclusions:** These data demonstrate independent effects of H and D on the impairment of suppression of HGO and the decrease in S_I, with D decreasing S_I independently of H, and H lowering the suppression of HGO, independently of D. The latter is not compensated by the peripheral hyperinsulinemia in HD and thus glucose tolerance deteriorates after hemipancreatectomy, regardless of pancreatic vein drainage.

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INSULIN ENHANCES GLUCOSE DISPOSAL DURING INTRAVENOUS GLUCOSE TOLERANCE TESTS IN THE RAT.

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Normalization of glucose during the intravenous glucose tolerance test (IVGTT) depends, in humans and dogs, in large part on the actions of secreted insulin to stimulate glucose disposal (R_d) and suppress endogenous glucose production (EGP). Rats, by contrast, exhibit rapid glucose dynamics during the IVGTT, and may be less dependent on insulin for maintaining glucose tolerance. To assess the importance of insulin in the rodent model, we performed IVGTTs in conscious rats (353 ±14 g) during somatostatin (SRIF, 6 μg/min per kg; n=6) or saline (n=10) infusion from t=–60 to 180 min. At time 0, cold glucose (0.3 g/kg), into which 3-^{3}H-glucose (20 μCi/kg) was added, was injected intravenously. Tracer and glucose dynamics (representing R_d and the combined actions on R_d and EGP, respectively) were quantified as the time to attain 50% of peak value ($t_{½}$). SRIF abolished basal insulin (< detectable limit of RIA), and inhibited dynamic insulin response by 70% (acute insulin response from 2 to 11 min; 148±58 vs. 513±130 μU/ml; p=0.025). Despite injection of identical tracer bolus (p=0.96), dynamics of labeled glucose were retarded 50% during SRIF ($t_{½}$: 18.6±1.6 vs. 12.5±1.1 min; p=0.011). Cold glucose dynamics also tended to be slower during IVGTTs with SRIF (15.1±3.3 vs. 10.7±1.0 min), although this trend was not significant (p=0.25). Glucose tolerance was also markedly reduced (59%) when insulin was suppressed (K_G: 1.74±0.19 vs. 4.33±0.38 min^{-1}; p=0.0001). These data indicate that glucose disposal in rats is sensitive to insulin during rapid dynamics of the IVGTT. Thus, both insulin sensitivity and glucose effectiveness (reflecting actions of glucose per se on disposal and glucose production) may be quantified in the conscious rat model.

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DIABETOGENIC ASSOCIATION OF IRS-1 Gly972→Arg AND IRS-2 Gly1057→Asp VARIANTS IN POLYCYSTIC OVARY SYNDROME AND OBESITY.

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Insulin resistance is a prominent feature of polycystic ovary syndrome (PCOS) and obesity. **Aims:** To understand how genetic defects in insulin action trigger glucose intolerance and NIDDM we investigated polymorphisms in insulin receptor, IRS-1 and IRS-2 genes in women with PCOS (n=41) or obesity (n=38). **Methods.** Polymorphisms were determined by gene sequencing on an *ABI373A* automatic sequencer and by screening with *SmaI* and *BanI* for Gly972→Arg of IRS-1 and Gly1057→Asp of IRS-2. **Results.** Glucose intolerant PCOS (BMI=32.3±1.2) or obese patients (BMI=45.3±1.4) displayed 57 and 16% *acanthosis nigricans* compared to 29% in normal tolerant PCOS, but none have receptor mutations. All glucose-intolerant PCOS displayed Gly1057→Asp variant of IRS-2 (86%) or double-mutations in IRS-2 and IRS-1 (14%). By contrast, 41% of normal glucose tolerant PCOS had none of IRS variants and displayed only 38% Gly1057→Asp of IRS-2, 17% double-mutations and 2.9% Gly972→Arg of IRS-1. Compared to non-mutated PCOS, Gly972→Arg of IRS-1 and Gly1057→Asp of IRS-2 induced 2- ($p<0.0002$) and 3-fold ($p<0.003$) increase in HOMA index in affected patients. Double-mutated PCOS patients had higher fasting and 2-h insulin levels during OGTT and were the most insulin resistant in both normal glucose tolerant (HOMA=5.1±0.3) or intolerant (HOMA=26.1) patients. Normal glucose tolerant obese had no IRS mutations in 46% and displayed 38.4, 7.6, and 7.6% mutations in IRS-2, IRS-1 and double-mutations, respectively. Strikingly 75% of glucose intolerant obese possess IRS-2 Gly1057→Asp or double-mutations IRS1/2 associated with higher fasting insulin (17 μU/ml, $p<0.0001$) and 2h insulin and glucose levels. **Conclusion.** These data indicated that allelic variants of IRS-2 in combination with IRS-1 are better correlated to glucose intolerance rather than simply to insulin resistance, and thus, may be used as predictive diabetogenic markers.

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TNF-α IS ASSOCIATED WITH IMPAIRED INSULIN-SIGNAL TRANSDUCTION FOLLOWING MUSCLE DAMAGE IN HUMAN SKELETAL MUSCLE.

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The physiological stress associated with muscle damage results in whole body insulin resistance and an acute phase immune response, leading to mononuclear cell release of cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-1. **Aims:** This study was conducted to determine the mechanisms of impaired insulin action, and whether cytokines play a role in the development of insulin resistance following muscle damage. **Materials and Methods:** To measure insulin-signal transduction, needle muscle biopsies were performed at baseline (BASE) and at 1 h during hyperinsulinemic (40 $mU \cdot kg^{-1} \cdot min^{-1}$) euglycemic (5.0 mM) clamps in 8 young (age 24±1 yr.) healthy sedentary (VO_{2max} 49.7±2.4 $ml \cdot kg^{-1} \cdot min^{-1}$) males without exercise (CTRL) and 24 h following eccentric exercise-induced muscle damage (ECC). In addition, venous blood samples were obtained before and 24 h following ECC to determine *in vitro* endotoxin-induced mononuclear cell (MNC) secretion of TNF-α, IL-6 and IL-1. **Results:** Glucose disposal rates (GDR) were lower ($P<0.05$) in the ECC compared to CTRL (3.9±0.7 vs 4.8±0.9 $ml \cdot kg^{-1} \cdot min^{-1}$). Insulin-stimulated IRS-1 tyrosine phosphorylation and Akt serine phosphorylation were lower ($P<0.05$) following ECC compared to CTRL (5.8±1.0 vs 3.2±1.3 and 25.0±5.9 vs 8.7±1.3 fold increase above BASE, respectively). Insulin-stimulated PI3-kinase activity and Akt activity were also lower ($P<0.05$) in the ECC trial (4.4±1.4 vs 2.9±1.3 and 1.5±0.1 vs 1.2±0.1 fold increase above BASE, respectively). TNF-α production, but not IL-6 or IL-1, was increased ($P<0.05$) 24 h following muscle damage (1.1±0.3 vs 2.6±0.9 $ng \cdot ml^{-1}$; pre-exercise vs. 24 h post-exercise). Furthermore, increases in TNF-α production were positively related to decreases in insulin-stimulated PI3-kinase activity ($r=0.77$, $P=0.04$). **Conclusions:** Therefore, the physiological stress associated with muscle damage impairs insulin-signal transduction, presumably leading to decreased insulin-mediated whole body glucose uptake. Furthermore, elevated production of TNF-α may be associated with downregulation of the insulin signaling pathway following muscle damage.

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TNF-α AND CELL-PERMEABLE CERAMIDE STIMULATE LIPOLYSIS IN 3T3-L1 ADIPOCYTES BY DOWNREGULATION OF PDE3B

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Lipolysis is regulated by the cAMP/protein kinase A cascade. Phosphodiesterase 3B plays a key role in determining the cAMP levels and thereby lipolysis in adipocytes; insulin-mediated stimulation of PDE3B results in lowering of cAMP/protein kinase A and inhibition of lipolysis. Increased adipocyte lipolysis by TNF-α promotes insulin resistance possibly through stimulation of free fatty acids, but the mechanim is not completely understood. TNF-α activates sphingomyelinase leading to production of ceramides. Ceramides mimic some effects of TNF-α on inhibiting insulin signaling. Aim: In this study, we hypothesized that the mechanism of TNF-α and ceramide-induced lipolysis might involve PDE3B. Material and Methods: 3T3-L1 adipocytes were treated with C_2-ceramide and TNF-α. Lipolysis and PDE3B activity, protein and mRNA were measured. Results: C_2-ceramide increased adipocyte lipolysis (5.5±0.9 μmol/mg protein/24h vs.1.8±0.2 in controls, $p<0.001$). Ceramide-induced lipolysis occured at 6h after treatment of cells and was significantly increased after 12h of incubation ($P<0.01$). Pretreatment of cells with C_2-ceramide and TNF-α blocked the ability of insulin to inhibit isoproterenol-induced lipolysis. Traglitazone (an antidiabetic agent of TZDs family) reduced ceramide-stimulated lipolysis by 90%, consistent with previously reported effects of TZD on TNF-α-induced lipolysis. Both C_2-ceramide and TNF-α decreased PDE3B activity by 50%, which was accompanied by a reduction of PDE3B protein and mRNA expression. C_2-ceramide resulted in elevation of cAMP levels by 20% ($p>0.05$). TNF-α increased protein kinase A activity by 75% ($p<0.001$). These data suggest that downregulation of PDE3B may contribute, at least in part, to the stimulation of lipolysis by ceramide and TNF-α in adipocytes. In addition, PD98059, Wortmannin and AG-490 (inhibitors of MAP kinase, PI-3 kinase and JAK-2 kinase) had no effect on ceramide-induced lipolysis and downregulation of PDE3B, thus suggesting that the effect of ceramides is independent of MAP kinase, PI-3 kinase and JAK kinase. Conclusion: Our study could provide a new insight into the mechanisms of insulin resistance.

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PREVALENCE OF INSULIN RESISTANCE IN RELATION TO AGE IN *PSAMMOMYS OBESUS*, A MODEL OF NUTRITONALLY INDUCED TYPE 2 DIABETES

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***Psammomys obesus* (desert gerbil, nicknamed "sand rat") was placed on a high energy (HE) diet at a young (8-20 wk) and old (38-45 wk) age. The young *Psammomys* progressed to severe insulin resistance, followed by pronounced hyperglycaemia and hyperinsulinaemia but the HE diet was non-diabetogenic at the high age. When young *Psammomys* received s.c. implants, releasing 2u/24 h insulin, there was no hypoglycaemia, fall in serum FFA or decline in hepatic phosphoenolpyruvate carboxykinase (PEPCK) activity despite serum insulin level of ~360 mu/l. However, in the aged *Psammomys* the exogenous hyperinsulinaemia considerably reduced within 5 h the serum glucose and FFA and suppressed the hepatic PEPCK. Euglycaemic-hyperinsulinaemic clamp revealed that the basal hepatic glucose production (HGP) was low in the aged *Psammomys* and was shut down (from 5.6±0.6 to 0.2±0.1 mg/min.kg) at the clamp insulin level of 280 mu/l. In contrast, in the young *Psammomys* HGP was reduced by 64% only (from 10.9±0.8 to 3.9±0.5 mg/min.kg). The effect of clamp insulin on total glucose transport was similar in the young and the old. Thus, HGP rather than the peripheral glucose underutilization was the main contributor to hyperglycaemia in the young and the hepatic insulin resistance became attenuated with age. Human implications: Prevalence of type 2 diabetes in Western society generally increases with age, whereas in high risk populations emerging from food scarcity into nutritional affluence, the diabetes prevalence tapers off with age. Therefore, the nutritionally induced diabetes in *Psammomys obesus* represents an appropriate model for the differing pattern of age related prevalence of diabetes.**

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THE PREVALENCE AND NATURE OF GLUCOSE INTOLERANCE IN KIDNEY TRANSPLANT RECIPIENTS

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Aim: the prevalence and nature of glucose intolerance was studied in 370 kidney transplant recipients. Among these patients 85 were known to have overt diabetes, and in 70 of them diabetes developed posttransplant. **Materials and Methods:** in the other 285 patients - having normal fasting plasma glucose at regular controls and no signs of diabetes - a 75 g oGT was performed. ICA and C-peptide was also measured from fasting blood samples. **Results:** based on the 2-h glucose values (WHO criteria) IGT was diagnosed in 80 (28.1%) and diabetes in 29 patients (10.2%). A fasting plasma glucose > 6.1 mM/L (ADA 1997) occured only in 13 patients of the IGT and 9 of the diabetic group. ICA was undetectable in each case. Mean fasting C-peptide level was 4,02±2,67 ng/ml in the diabetic group and 5,32±2,80 ng/ml in 22 non-diabetic transplanted patients, both exceeding significantly ($p<0.001$) that of 24 healthy control subjects (1,50±0,45 ng/ml). **Conclusions:** posttransplant disturbances in glucose metabolism are more frequent than have been suggested by literature data. However, in the majority of cases only "isolated postchallenge hyperglycemia" was found. Fasting plasma glucose is of little diagnostic value in posttransplant glucose intolerance. This condition is characterized by insulin resistance as evidenced by elevated C-peptide levels.

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ADDITIVE STIMULATORY EFFECT ON INSULIN AND LEPTIN GLUCOSE TRANSPORT IN ISOLATED RAT HEARTS.

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We have preveously shown in C_2C_{12} myotubes, that leptin at low physiological concentrations (1ng/ml) is able to stimulate glucose transport and glycogen synthesis through JAK-2-, IRS-2- dependent stimulation of PI3-kinase. Since other investigators were not able to find a direct effect of leptin in various skeletal muscle preparations, we hypothesised that such a leptin effect might be important only in muscle requiring constant glucose supply independant of insulin such as heart or diaphrama. We therefore studied the effect of leptin and insulin on glucose transport in isolated hearts from male Wistar rats perfused in a constant pressure Langendorff preparation with simultaneous measurement of hemodynamic function. Glucose transport was assessed in tracer washout experiments using the non-metabolizable glucose analog ^{3}H-3- O-methyl-glucose (3-O-MG) and the non-transportable tracer ^{14}C-D-Mannitol in equimolar concentrations. For quantitative interpretation of tracer kinetics a simplified 3-compartiment mathematical model was used with extracellular space (vascular-space) as first, intracellular space as second and the perfusion-buffer as third compartiment. Hearts were perfused with insulin (10^{-9}M), Leptin (1 ng/ml) or the combination of both hormones at concentrations which did not affect coronary flow.

n = 10 each group	glucose transport (k_{in} min^{-1})	increase (%) treatment vs control	mannitol transport (k_{in} min^{-1})	increase (%) treatment vs control
controls	0.23	----	0.23	----
insulin	0.49	116*	0.23	2
leptin	0.29	28	0.20	-10
ins.+lept.	0.54	142*	0.23	1

* $p<0.05$ vs controls

These data suggest that leptin at physiological concentrations exerts a partial insulin like effect on glucose transport. The effect seems to be additive to insulin implicating a different mechanism in stimulating glucose uptake. Leptin might contribute to insulin independent basal glucose supply of the myocardium.

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ACE INHIBITION AND GLUCOSE TRANSPORT IN INSULIN-RESISTANT RAT MUSCLE: ROLES OF BRADYKININ AND NITRIC OXIDE.

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Aims: We have shown previously that acute in vivo administration of the angiotensin converting enzyme (ACE) inhibitor captopril enhances insulin-stimulated glucose transport activity in isolated skeletal muscle of the insulin-resistant obese Zucker rat. The present study was designed to assess whether this effect is mediated by increased action of the nonapeptide bradykinin (BK), by decrease action of angiotensin II (ATII), or by both factors. **Materials and Methods:** Obese Zucker rats (8-9 wk old) were treated over 2 hr with either captopril (50 mg/kg p.o.), BK (200 μg/kg i.p.), or the ATII receptor (AT1 subtype) antagonist eprosartan (20 mg/kg p.o.). **Results:** Captopril treatment enhanced in vitro insulin-stimulated (2 mU/ml) 2-deoxyglucose uptake in the epitrochlearis muscle by 22% (251 ± 7 pmol/mg/20 min vs. 205 ± 9, $p<0.05$), while BK treatment enhanced this variable by 18% (249 ± 15 vs. 215 ± 7, $p<0.05$). Eprosartan administration did not significantly modify insulin action. The BK-mediated increase in insulin action on skeletal muscle glucose transport was completely abolished by pre-treatment with either the specific BK-B_2 receptor antagonist HOE 140 (200 μg/kg i.p.) or the nitric oxide synthase inhibitor, Nω-nitro-L-arginine methyl ester (L-NAME, 50 mg/kg i.p.). **Conclusions:** Collectively, these results indicate that the modulation of insulin action on skeletal muscle glucose transport activity by BK likely underlies the metabolic effects of ACE inhibitors in the insulin-resistant obese Zucker rat. Moreover, this modulation of insulin action by BK is likely mediated through BK-B_2 receptors and also by an increase in nitric oxide production and/or action in skeletal muscle tissue.

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DEHYDROEPIANDROSTERONE EXERTS DISTINCT REGULATORY EFFECTS ON THE GLUCOSE TRANSPORT SYSTEM IN FAT AND MUSCLE CELLS.

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Plasma dehydroepiandrosterone (DHEA) levels correlate with whole-body insulin sensitivity in humans, and DHEA administration ameliorates insulin resistance in experimental obesity and diabetes. However, the direct effects of DHEA on glucose uptake by specific cell types have not been investigated. Aims of this study were to compare DHEA effects on glucose transport in adipocytes and skeletal muscle cells and to define the mechanisms of DHEA regulation. Results. DHEA (1-100 μM for 2 h) induced a dose-dependent increase in 2-deoxyglucose transport in 3T3-L1 adipocytes (up to 240% of control, $P<0.05$), and this was associated with increased plasma membrane levels of GLUT1 and GLUT4 transporters (respectively 200% and 180% of control, $P<0.05$). Similar results were obtained also in isolated human adipocytes. Glucose transport stimulation by DHEA was not affected by pre-treatment of adipocytes with the PI 3-kinase inhibitors wortmannin (200 nM) and LY294002 (50 μM), that blocked transport stimulation by insulin. By contrast, DHEA effects on GLUT1/4 translocation and glucose transport were abrogated in the presence of 20 μM GF109203X, a general protein kinase C (PKC) inhibitor, and 50 nM Gö6976, a selective inhibitor of Ca^{2+}-dependent PKC isoforms. Insulin stimulation of transporter translocation and glucose transport was partially inhibited by GF109203X (by 50%, $P<0.05$) and not changed by Gö6976. In L6 myocytes, DHEA induced a 2.5-fold increase in plasma membrane GLUT1 and GLUT4, as in 3T3-L1 adipocytes, and this effect was also inhibited by GF109203X. Surprisingly, in spite of increased cell-surface transporters, 3-O-methylglucose and 2-deoxyglucose transport rates were markedly decreased in DHEA-treated L6 myocytes (45% and 15% of control, $P<0.05$). A similar decrease was also observed in isolated mouse soleus muscle incubated with DHEA in vitro (28% of control, $P<0.05$). The ability of DHEA to decrease glucose transport in skeletal muscle cells was preserved in the presence of inhibitors of PI 3-kinase, PKC, pp70S6 kinase, or MAP kinase. Conclusions. DHEA promotes GLUT1 and GLUT4 translocation to the cell surface in both adipocytes and myocytes via Ca^{2+} dependent PKC isoforms and, thus, utilizes a signaling pathway distinct from insulin. The DHEA-mediated increase in cell-surface transporters results in enhanced glucose transport rates in adipocytes, but not in myocytes because DHEA appears to suppress transporter catalytic activity in this cell type via a novel signaling mechanism.

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PROTEIN-TYROSINE-PHOSPHATSE 1D IS PHOSPHORYLATED BY PROTEIN KINASE C ISOFORMS α, β1 AND β2.

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Aim: Serine kinases like protein kinase c (PKC) and protein tyrosine phosphatases (PTP) are potential modulators of the insulin signalling chain and might play a role in the induction of cellular insulin resistance. It is speculated that insulin receptor (IR) and IRS-1 inhibition by PKC involve PTP activation. A serine phosphorylation of PTP-1C by PKC was observed while nothing is known about the interaction of PKC with the ubiquitously expressed PTP-1D. The aim of the present study was to investigate the interaction of PKC-isoforms with the PTPases 1C/1D with particular respect to the involvement of IR/IRS-1. **Methods**: HEK293 cells were cotransfected with PKC isoforms (α, β1, β2, γ, δ, ε, ζ, θ, η, ι), PTP-1C or 1D, IR and IRS-1. Cells were incubated with or without insulin (100nM, 5 min), TPA (100nM, 30 min) or with the PKC inhibitor bisindolylmaleimide (BIM, 200nM, 60 min) and immunoblots were detected with anti-PY or respective proteins. PTP-activity was measured in a pNPP dephosphorylation assay. **Results**: We could show a defined TPA induced mobility shift of PTP-1D in PKC α, β1, or β2 cotransfections which is not obtained with the kinase inactive forms of PKC β1 and β2. The effect could be completely blocked with the PKC inhibitor BIM. We observed that this shift does not occur with other PKC isoforms or PTP-1C and that it is not dependent on IR or IRS-1 overexpression. First activity studies revealed no differences in PTP-1D activity with or without TPA preincubation. Additionally the inactive mutant of PTP-1D altered the phosphorylation pattern. **Conclusions**: The data suggest an interaction between PTP-1D and PKC α, β1, or β2. It remains unclear which phosphorylation sites are responsible for the mobility shift. Consequences for cellular localization and PKC dependent IR and IRS-1 phosphorylation are currently investigated.

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PHOSPHOPROTEIN ENRICHED IN DIABETES (PED) AND RAS ASSOCIATED WITH DIABETES (RAD) ARE OVEREXPRESSED IN FIBROBLASTS (F) FROM A PATIENT WITH A SYNDROME OF SEVERE INSULIN RESISTANCE ASSOCIATED WITH DEFECTIVE INSULIN-STIMULATED GLUCOSE TRANSPORT.

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Aims: It has recently been shown that protein and gene expression of PED/PEA-15, a PKC-substrate, is increased by ~65% in F derived from patients with type 2 diabetes. Furthermore transfection of PED/PEA-15 in L6 muscle cells inhibited insulin-stimulated glucose transport (ISGT) (EMBO J.14:3858,(1998)). To extend these investigations, the aim of the current study was to determine PED/PEA-15 expression in F derived from a patient with severe insulin resistance associated with defective ISGT. In addition, the expression of RAD, a member of the Ras-related small GTPases, whose role in the mole-cular pathogenesis of decreased ISGT in type 2 diabetes is controversial, was also detemined.

Methods and results: When compared to nondiabetic controls and patients with type 2 diabetes PED/PEA-15 protein expression was increased by 332% ($p<0.01$) and 72% ($p<0.05$) respectively. Cytosolic RAD protein expression was not statistically different in controls and type 2 diabetics. In contrast, in F from the insulin resistant patient, RAD expression was increased by 275% when compared to both controls and patients with type 2 diabetes.

Conclusion: In F derived from a patient with severe insulin resistance associated with defective ISGT, both PED/PEA-15 and RAD proteins were markedly increased when compared to controls as well as type 2 diabetic individuals, emphasizing the role of these proteins and the PKC-signalling pathway in the molecular pathogenesis of defective ISGT.

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Insulin Signalling

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MUSCLE FIBRE-TYPE SPECIFICITY IN INSULIN SIGNAL TRANSDUCTION

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Aims: Skeletal muscle fibres are classified into distinct categories: type I (slow-twitch-oxidative), type IIa (fast-twitch-oxidative-glycolytic) and type IIb (fast-twitch-glycolytic). We determined the muscle fibre-type specific response of intracellular signalling proteins including the insulin receptor, IRS-1, PI 3–kinase and Akt kinase to insulin. **Methods** Epitrochlearis (EPI; 15% type I, 20% type IIa and 65% type IIb), soleus (84-16-0%) and extensor digitorum longus (EDL; 3-57-40%) muscles were obtained from Wistar rats and incubated in the absence or presence of 120 nmol/l insulin (3-40 min). **Results:** Peak IR tyrosine phosphorylation was reached after 6 (soleus) and 20 (EPI and EDL) min insulin exposure, with sustained activity throughout the remaining insulin exposure (40-min). Insulin increased IRS-1 tyrosine phosphorylation and phospho-tyrosine-associated PI 3-kinase activity to a maximal level after 6-10 min, with a subsequent down-regulation after 10-20 min. Insulin-stimulated Akt kinase phosphorylation peaked at 20 min and was sustained throughout the insulin exposure. Soleus muscle demonstrated the greatest insulin response for all signalling intermediates tested. Immunoblot analysis of IRS-1, p85α subunit of PI 3-kinase, and Akt revealed these proteins were expressed to a greater extent in oxidative (soleus) versus glycolytic muscle (EPI and EDL).**Conclusions:** Insulin-signal-transduction is greatest in skeletal muscle composed primarily of oxidative fibres. Increased expression of key proteins in the insulin-signalling cascade appear to account for fibre-type specific differences in insulin-stimulated glucose uptake and metabolism in skeletal muscle. Increasing the oxidative capacity of skeletal muscle may be one therapeutic strategy to overcome defective insulin-signal-transduction in insulin resistant skeletal muscle.

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EFFECTS OF EXERCISE-TRAINING ON INSULIN-SIGNALLING IN SKELETAL MUSCLE

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Aims: Level of physical activity is linked to improved glucose homeostasis. We determined whether exercise-training alters insulin-signalling in skeletal muscle. **Methods** Female Wistar rats performed two 3-hour swim bouts, for 1 or 5 days. Epitrochlearis muscle were excised 16 hours after the last exercise bout, and incubated +/- insulin (120 nM). **Results:** Insulin-stimulated glucose transport was 30 and 50% higher after 1 and 5 day training ($P<0.05$). Insulin-stimulated receptor tyrosine phosphorylation was not increased until 5-day training (2-fold; $P<0.05$). Protein expression of GLUT 4 and insulin receptor increased 2-fold after 1-day, with no further change after 5-day training. IRS-1 protein content decreased 50% after 5-day training ($P<0.05$). In contrast, insulin-stimulated IRS-1 tyrosine phosphorylation and IRS-1 associated PI 3-kinase activity increased 2.5-fold and 3.5 after 1 and 5-day training respectively. IRS-2 protein content increased 2.6-fold after 1-day ($P<0.05$), and normalised to sedentary levels after 5-day training. Basal and insulin-stimulated IRS-2 associated PI 3-kinase activity increased 2.8-fold ($P<0.05$) and 9-fold ($P<0.05$) respectively, after 1-day and normalised to sedentary levels after 5-day training. Insulin-stimulated Akt phosphorylation increased 5-fold after 5-day training ($P<0.05$), whereas MAP Kinase phosphorylation was not altered by training **Conclusions:** Collectively, these proteins undergo dynamic regulation in response to exercise. Thus, exercise training may be an effective strategy to overcome reduced insulin-signaling in skeletal muscle from Type 2 diabetic patients.

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EFFECTS OF MUSCLE CONTRACTIONS ON GLUCOSE METABOLISM AND ACTIVATION OF THE MAP KINASE CASCADE

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Aims: Signalling cascades leading to glucose transport and the Map kinase signalling pathways are known to be activated in response to muscle contraction. Our aim was to determine the extent to which contraction activates glucose metabolism and MAP kinase cascades in skeletal muscle. **Methods:** Rat epitrochlearis muscles were excised from Wistar rats and forced to contract by *in vitro* electrical stimulation. Two different electrical stimulation protocols (Stim 1: 1 contraction/2 sec and Stim 2: 1 contraction/ min) were used for a total of 10 min. **Results:** Basal glucose transport (0.93±0.06 μmol/ml/h) was increased 6-fold ($p<0$-01) with stimulation (5.86±0.63 or 4.63±0.31 μmol/ml/h for Stim 1 and Stim 2, respectively). Insulin (120 nM) led to a similar increase in glucose transport (6.43±0.99 μmol/ml/h). Glycogen content (24.6±1.2 mmol/g wet wt) was markedly reduced by 70% ($p<0.01$) after each Stim protocol (9.4±0.4 *vs.* 9.1±1.4 mmol/g wet wt for Stim1 *vs.* Stim 2), with no change after insulin exposure. Electrically induced contraction led to a 3 or 2- fold increase in p42/44 Map kinase phosphorylation for Stim 1 or Stim 2 respectively ($p<0.05$). Stim 1 and Stim 2 increased p38 phosphorylation 40 and 80 fold respectively, with insulin stimulation showing no effect. Both stimulation protocols resulted in 4-fold activation of mitogen and stress activated protein kinase (MSK) 1 and 2 ($p<0.05$). **Conclusions:** Muscle contraction directly activates intracellular kinases. Activation of these kinases has been linked to cell proliferation and differentiation. MAP kinase activated signalling cascades may link muscle contractions to alterations in gene expression.

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NEW HUMAN INSULIN ANALOGS: CHARACTERISTICS OF INSULIN SIGNALLING IN COMPARISON TO ASP(B10) AND REGULAR INSULIN

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Aim: Amino acid exchange of insulin is a useful strategy to create new insulin analogs with improved pharmacokinetic properties. On the other hand mutations of the insulin gene have the potential to induce altered metabolic and mitogenic effects like the insulin analog Asp(B10) which is known to have increased mitogenic activity. The aim of the study was to compare HMR 1153→Lys(B3),Ile(B28); HMR 1964→Lys(B3),Glu(B29) and HMR 1423→Gly(A21),His(B31),His(B32) with normal human insulin and the analog Asp(B10). **Methods:** We have analyzed receptor binding, insulin-induced receptor auto- and dephosphorylation as well as phosphorylation of the insulin receptor substrate in rat-1 fibroblasts overexpressing the human insulin receptor isoform B (HIR B). **Results:** In HIR B expressing cells, insulin and its new analogs showed no significant differences in receptor association and dissociation while clearly different association kinetics were observed with Asp(B10). All insulins induced rapid autophosphorylation of the insulin receptor reaching a maximum after 10 min of stimulation with 10^{-9} M insulin. Asp(B10)insulin induced a prolonged phosphorylation and dephosphorylation state of the 95kDa receptor ß-subunit and the IRS-proteins. With respect to [^{3}H]thymidine incorporation into DNA, the new analogs had similar effects as normal human insulin, while Asp(B10)insulin showed increased [^{3}H]thymidine incorporation. **Conclusions:** The analogs HMR 1153→Lys(B3),Ile(B28); HMR 1964→Lys(B3),Glu(B29) and HMR 1423→Gly(A21),His(B31), His(B32) show the same binding and signalling characteristics compared to normal human insulin and might be useful tools for the treatment of diabetes.

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GROWTH PROMOTING ACTIVITY OF INSULIN ASPART AND OTHER INSULIN ANALOGUES

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Aim: Insulin binding to the insulin receptor mediates both metabolic and mitogenic effects of the hormone. At supra-physiological insulin concentrations, insulin can also promote cell-growth via the IGF-1 receptor to which insulin binds with low affinity. We have examined the IGF-1 receptor affinity and mitogenic potential of the novel rapid-acting insulin analogue insulin aspart (B28Asp) in comparison to that of human insulin (HI) and reference insulin analogues. Methods: HepG2 cells or solubilised human IGF-1 receptors were used in binding experiments. Mitogenicity studies were done using human mammary cancer fibroblasts (MCF-7 cells; end-point:% cells in S-phase) or B10 human osteosarcoma cells (end-point: ^{3}H-thymidine incorporation). Results are presented with 95% c.i. or as mean±SE. Results: Insulin aspart and HI both showed more than 1000-fold lower affinity than IGF-1 for solubilised IGF-1 receptors. The IGF-1 receptor affinity of insulin aspart was determined to 68.8% (62.1-76.2%) relative to HI (=100%) using HepG2 cells. In comparison, insulin X10 (B10Asp) binds with 4-fold greater affinity to the IGF-1 receptor, and the IGF-1 receptor affinity (HepG2 cells) of insulin lispro (B28Lys, B29Pro) was determined to 141.5% (130.6-153.4%) in accordance with literature data. The mitogenic potency of insulin aspart was similar to that of HI (=1.0) in both MCF-7 cells: 1.2 (0.9-1.6) and in B10 cells: 0.58±0.22. In comparison the mitogenic potential (B10 cells) of insulin lispro was 0.66±0.21, and that of insulin X10 was more than 10-fold greater. The equivalent mitogenic potential of insulin aspart and HI is in agreement with previous results in non-human cells (CHO-K1 and rat aortic smooth muscle cells). Conclusion: IGF-1 receptor affinity and insulin receptor dissociation rate have recently been discussed in relation to mitogenic and tumourigenic potential of insulin analogues. Insulin aspart has previously been shown to be equivalent to HI in dissociating from the insulin receptor. As shown herein, the IGF-1 receptor affinity and mitogenic potential of insulin aspart are also equivalent to that of HI, supporting that the non-IGF-1 like amino acid substitution B28Asp is safe.

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INSULIN RECEPTOR TYROSINE KINASE ACTIVATION BY INSULIN ASPART AND INSULIN ANALOGUES WITH A WIDE RANGE OF RECEPTOR BINDING AFFINITIES

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Aims: Activation of the insulin receptor tyrosine kinase (IRTK) is essential for many if not all of the biological effects of insulin. However, no studies have attempted to systematically study structure-function relationships for activation of the IRTK. We have examined the correlation between insulin receptor binding and activation for a variety of insulin analogues. More comprehensive studies were done to examine the insulin receptor binding and activation properties of insulin aspart (B28Asp), a novel rapid acting insulin analogue. Methods: HepG2 cells or solubilised human insulin receptors were used for receptor binding experiments. IRTK activation was determined using receptors partly purified from rat skeletal muscle or using CHO-hIR cells. ^{32}P incorporation into poly-Glu-Tyr was used as end-point. Results are presented with 95% c.i. or as mean±SE. Results: Insulin receptor affinities ranged from 7.5% to 687% (HepG2 cells) and potencies for IRTK activation ranged from 16% to 681% (rat IRTK) relative to human insulin (HI = 100%). There was a strong linear correlation (r=0.95, n=21) between receptor binding and activation, supporting the notion that IRTK activation is closely linked to receptor binding. Relative insulin receptor binding affinities for insulin aspart were 92±6% using solubilised receptors and 92% (82.0-103.7%) using HepG2 cells. Insulin aspart activated both the rat (100±19%) and human (107±32%) IRTK with equivalent potency as HI (=100%). In comparison, insulin X10 (B10Asp) was 2-7 fold more potent in binding and activating the insulin receptor in the applied models and insulin lispro (B28Lys, B29Pro) showed an insulin receptor affinity (HepG2 cells) of 101.7% (96.6-107.1%) and a potency for human IRTK activation (CHO-hIR cells) of 82±24%. Conclusion: The results indicate that insulin aspart is equivalent to human insulin in binding and activating the insulin receptor, and that IRTK activation is strongly correlated to insulin receptor occupancy for insulin analogues with a wide range of receptor affinities.

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INSULIN-INDUCED ACTIVATION OF JNK IS REGULATED BY SHP-2

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Aims: SHP-2, a protein tyrosine phosphatase with two SRC homology 2 domains, is believed to participate in signal relay downstream of insulin receptor. SHP-2 also plays an important role in control of cytoskeltal architecture. Recent studies suggest that insulin activates c-JUN NH2-terminal kinase (JNK) which activation requires intact cell structure. Therefore we have investigated whether SHP-2 regulates insulin-stimulated JNK activation.

Material and Methods: We established Rat-1 fibroblasts overexpressing human insulin receptors (Rat-1-IR) and Rat-1-IR that overexpresses a dominant-negative mutant SHP-2 (Rat-1-IR SHP-2C/S). The insulin effect on JNK activation was examined in these cells.

Results: In this report, we show that insulin treatment induced a time-dependent activation of JNK in Rat-1-IR while this was markedly reduced in mutant cells. In contrast, JNK activation by anisomycin, solbitol and UV treatment was not reduced in mutant cells. PI-3-kinase inhibition by wortmannin completely blocked insulin activation of JNK. Dissembly of the actin filaments by cytochalasin D reduced insulin-stimulated JNK activation in Rat-1-IR, but partially recovered the ability of insulin to stimulate JNK in mutant cells, suggesting that actin filament network plays an essential role in SHP-2 regulation of insulin-stimulated JNK activation pathway.

Conclusions: Our results demonstrate that SHP-2 may modulate the action of insulin on JNK via cell structure regulation.

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Indinavir decreases insulin-stimulated insulin receptor substrate-1 phosphorylation in human Hep G2 cells

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Aims: Patients receiving human immunodeficiency virus type 1 protease-inhibitors (PI) are at risk to develop diabetes or impaired glucose tolerance, most probably due to an induction of insulin resistance by the PI. To explore the mechanism by which PI might effect insulin action we investigated whether treatment with the PI indinavir (gift from Merck & Co) alters insulin signaling. **Materials and Methods:** Hep G2 cells were first incubated in the presence or absence of indinavir (100 µmol/l) for 48 hours, then exposed to 0 (-I) or 100 (+I) nmol/l insulin for 75s and rapidly frozen. Frozen cells were solubilized, IRS-1 immunoprecipitated and Western blots performed with anti-IRS-1 or anti-phosphotyrosine antibody. The amount of IRS-1 phosphorylation and IRS-1 protein were analyzed by densitometry and results expressed as densitometric units (DU). **Results:** The insulin effect on IRS-1 phosphorylation was lower in cells with indinavir incubation (-I: 0,06±0,03; +I: 1,51±0,10 DU) than in cells without prior indinavir incubation (-I: 0,08±0,04; +I: 2,35±0,15 DU; $p \leq 0,05$). This indinavir effect was not explained by an alteration in the amount of IRS-1 protein (-I: 0,67±0,29; +I: 1,22±0,13 DU, and -I: 0,81±0,14; +I: 1,29±0,15 DU in cells pretreated with and without indinavir, respectively). Indinavir had also no effect on cell number or cell viability as evaluated by trypan blue exclusion and cell counting. **Conclusions:** Our results indicate that decreased insulin-stimulated IRS-1 phosphorylation might cause or contribute to the metabolic effects of PI-treatment.

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SIGNALING OF AN EPIDERMAL GROWTH FACTOR-INSULIN RECEPTOR CHIMERA WITH Y1158F MUTATED REGULATORY LOOP

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Aim: the insulin receptor (IR) is a member of the tyrosine kinase receptor family. The IR induces mitogenic as well as pronounced metabolic responses (i.e. anti-lipolytic action, glucose uptake and glycogen synthesis). The mechanisms which give rise to the diversity in signaling are largely unknown. Phosphorylated tyrosines within the cytoplasmic portion of the IR are involved in the binding of a variety of substrate proteins, at least partially leading to the pleiotropic signaling. Y1158 within the regulatory loop of the IR is involved in the specific binding of the phosphotyrosine-specific phosphatase 1D (SHP2). Y1158, in combination with Y1162 and Y1163 of the IR regulatory loop are important for binding and phosphorylation of insulin-receptor-substrate-2 (IRS2). The aim of the study was to determine the molecular basis of the diversity in signaling. **Material and Methods**: the intracellular region of the EGFR was exchanged with the IR counterpart, creating a chimeric receptor (EIR). Tyrosine phosphorylation sites of the IR regulatory loop within the chimera were exchanged for fenylalanine residues. Chimeric receptors were expressed in 3T3-L1 pre-adipocytes, which do not show metabolic responses upon EGF stimulation. After EGF stimulation, receptor specific signaling was examined by measurement of insulin-specific responses (i.e. IRS-1/2 phosphorylation, glucose uptake and glycogen synthesis). **Results:** we found that, upon EGF stimulation, the chimera EIR stimulates glycogen synthesis with a dose response relation comparable to the wt-IR. A chimeric receptor with exchanged Y1158 (EIR-Y1158F) gives rise to a bell shaped curve of glycogen synthesis. Glycogen synthesis collapses at hormone concentrations over 10^{-9} M. At low hormone concentrations (10^{-10} M EGF) the glycogen synthesis is normal and comparable with wt-IR and EIR. The chimera EIR-Y1158F shows an impaired phosphorylation of IRS-1 and IRS-2. **Conclusion:** the results suggest that Y1158 in the regulatory loop of the insulin receptor is important for a correct phosphorylation of IRS proteins, and a normal induction of metabolic responses.

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Thiazolidinediones

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ARE THE EFFECTS OF THIAZOLIDINEDIONES ON ISOLATED RAT MUSCLE INDEPENDENT OF GENE TRANSCRIPTION?

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Aims: Thiazolidinediones (TZD) are believed to exert their antidiabetic effects via modulation of gene transcription and protein synthesis as induced by agonistic interaction with nuclear peroxisome proliferator-activated receptor-γ (PPARγ). The present study examines whether the same mechanism of action underlies TZD-induced inhibition of CO_2 production from palmitate in isolated rat muscle. **Materials and Methods:** Freshly isolated rat soleus muscle strips from Sprague-Dawley rats were incubated in the presence of the TZD compounds troglitazone or rosiglitazone, or in the presence of the retinoid X receptor (RXR) agonist LG-100.268. CO_2 production from palmitate was measured under insulin-stimulated conditions. **Results:** Exposure to 20 μM troglitazone significantly inhibited CO_2 production from palmitate within 90 min (nmol palmitate into CO_2/g/h: control, 124±8 *vs* troglitazone 56±4, p<0.0001). The conversion of palmitate to CO_2 was also inhibited by 24 h exposure to 5 μM of each employed compound (nmol palmitate into CO_2/g/h: control, 95±5, *vs* troglitazone, 33±3, *vs* rosiglitazone, 74±7, *vs* LG-100.268, 62±5; p *vs* control <0.005 each), whereby the superior efficacy of troglitazone *vs* rosiglitazone contrasts with the compounds' respective antidiabetic potentials as well as with their potentials to activate PPARγ-dependent gene transcription (rosiglitazone> troglitazone). **Conclusions:** The efficacy of both, TZDs and LG-100.268, hints at inhibition of CO_2 release from palmitate via PPARγ/RXR hybrid receptors. On the other hand, rapid occurence of the response and the relative superiority of troglitazone over rosiglitazone suggest that direct TZD action *in vitro* is independent of PPARγ /RXR induced modulation of gene transcription rates. We conclude that the direct effects of TZD on muscle fuel metabolism *in vitro* are mediated via a previously unknown, transcription-independent pathway that is sensitive to PPARγ agonists as well as to RXR agonists.

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INHIBITION OF RAT SKELETAL MUSCLE FUEL OXIDATION BY THIAZOLIDINEDIONES *IN VITRO*.

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Aims: The study examines the direct effects of insulin sensitizing thiazolidinediones (TZD) on skeletal muscle, the quantitatively most important insulin target tissue. **Materials and Methods:** Freshly isolated rat soleus muscle strips from Sprague-Dawley rats were incubated for 24h in the presence of 5μM TZD, and parameters of basal or 100nM insulin stimulated fuel metabolism were measured. **Results:** As compared to controls, the TZD troglitazone markedly inhibited the rates of CO_2 production from glucose and palmitate (nmol glucose into CO_2/g/h: basal, 1256±90 *vs.* 621±38, p<0.001; insulin stimulated, 1461±753 *vs.* 753±80, p<0.0001; nmol palmitate into CO_2/g/h: basal, 80±8 *vs.* 24±2., p<0.0001; insulin stimulated, 75±5 *vs.* 20±2, p<0.0001). Inhibition of oxidative substrate utilization was associated with reduced rates of glycogen synthesis presumably caused by enhanced glucose requirements for anaerobic glycolysis (μmol glucose into glycogen/g/h: basal, 1.40± 0.11 *vs.* 0.87±0.10, p<0.0001; insulin stimulated, 2.00±0.26 *vs.* 1.02±0.13., p<0.001; μmol lactate released/g/h: basal, 12.6±0.7 *vs.* 39.3±4.4, p<0.0001; insulin stimulated, 17.3±1.0 *vs.* 49.2±2.7, p<0.0001). The same effects were found in insulin resistant muscle from genetically obese Zucker (fa/fa) rats (insulin stimulated: nmol glucose into CO_2/g/h, 541±103 *vs.* 182±19, p<0.02; nmol palmitate into CO_2/g/h: 15.7±1.2 *vs.* 8.1±1.0, p<0.002). Furthermore, inhibition of fuel oxidation was not specific for troglitazone, because it also occurred in response to other TZD compounds (control *vs.* BM13.1258 *vs.* BM15.2054, insulin stimulated: nmol glucose into CO_2/g/h: 1613 ±146 *vs.* 601±49 *vs.* 848±74; nmol palmitate into CO_2/g/h: 89±7 *vs.* 43±5 *vs.* 42±4; p<0.001 each *vs.* control). **Conclusions:** TZD directly inhibit fuel oxidation in isolated rat skeletal muscle independent of insulin stimulation and of prevailing insulin resistance. The clinical relevance of such previously unknown direct TZD action on skeletal muscle awaits to become clarified.

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ROSIGLITAZONE PLUS β3-ADRENOCEPTOR AGONIST TREATMENT NORMALISES GLYCAEMIA IN DIABETIC C57BL/KsJ *db/db* MICE

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Aims: To investigate the combined effects of the PPARγ agonist rosiglitazone (RSG) and the β3-adrenoceptor agonist BRL-35135 (BRL) on long-term glycaemic control in *db/db* mice. **Materials and Methods:** Female C57Bl/KsJ diabetic *db/db* and nondiabetic *db/+* mice were grouped according to non-fasting blood glucose levels at 9 weeks of age. Treated animals received either a sub-maximally effective dose of RSG (30 umol/kg diet) or BRL (10 umol/kg diet) as monotherapy or both doses combined. **Results:**

Non-fasting blood glucose concentration (mmol/l) after 78 days of treatment

(Group size was 9 or 10)	Control *db/db*	*db/db* + RSG	*db/db* + BRL	*db/db* +RSG&BRL	Control *db/+*
mean	28.7	9.0	13.0	7.6	6.5
95% con limits	24.3-33.8	7.7-10.7	11.0-15.3	6.4-9.0	5.5-7.8

In *db/db* mice, both RSG and BRL as monotherapy significantly reduced blood glucose concentrations compared to the untreated diabetic controls (P<0.001). In combination the two agents produced an additional glucose-lowering effect such that the blood glucose was reduced to levels measured in lean untreated *db/+* control animals (P>0.2). Combination treatment also significantly reduced terminal non-fasting plasma triglyceride concentration by 34% (control *db/db*: 1.35 (1.16-1.58) mmol/l vs. RSG&BRL *db/db*: 0.89 (0.76-1.04) mmol/l, P<0.001) and non-esterified fatty acid concentration by 48% (control *db/db* 1.64 (1.37-1.96) mmol/l vs. RSG&BRL *db/db*: 0.86 (0.72-1.03) mmol/l, P< 0.001) (geometric mean (95% CL), n=8). **Conclusions:** Both RSG and BRL alone were effective at establishing long-term glycaemic control in the diabetic mouse, and in combination they achieved complete normalization of blood glucose concentrations and lowered plasma lipids. These findings suggest that the complementary actions of RSG and a β3-adrenoceptor agonist may provide novel benefits in the treatment of type 2 diabetes.

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INSULIN-SENSITISING ACTION OF ROSIGLITAZONE IS ENHANCED BY FOOD RESTRICTION

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Aims: We investigated if food restriction would potentiate the insulin-sensitising effect of high dose rosiglitazone (RSG) in dietary obese (DIO) rats and studied mechanisms of associated weight gain. **Materials and Methods**: Obesity was induced in male rats by feeding a palatable diet for 10 wks. (575±10 *vs* 536±7 g in chow-fed controls; $p<0.01$). RSG (30 mg/kg p.o. daily) or vehicle were given for 14 days. Half of the RSG-treated group were pair-fed to *ad lib*-fed DIO controls. **Results**: Food intake was increased by RSG in *ad lib*-fed DIO rats (8098±209 *vs* 6861±132 kJ; $p<0.0001$). RSG normalised insulin sensitivity in DIO rats (HOMA: chow controls, 3.9±0.3; DIO, 6.7±0.7; RSG-treated *ad lib* DIO, 4.2±0.5; both $p<0.01$), and was further improved by calorie restriction (2.9±0.4; $p<0.05$ *vs* RSG-treated *ad lib* DIO). Plasma free fatty acids (FFA) were higher in DIO control rats (1.3±0.2 *vs* 0.8±0.1 mmol/l, $p<0.001$), lower after RSG (0.17±0.03mmol/l, $p<0.01$), but not further reduced by food restriction (0.23±0.04 mmol/l). Weight gain was greater in DIO rats than in controls (34±2 *vs* 24±2 g) and was still greater after RSG (74±7g, $p<0.001$), even when animals were calorie restricted (49±5g, $p<0.001$). Adipose tissue leptin mRNA and plasma leptin were increased in DIO rats (7.8±0.5 *vs* 4.8±0.4 ng/ml; $p<0.0001$), and fell with RSG administration (7.1±0.6 ng/ml; $p=0.05$) and calorie restriction (5.8 ± 0.5ng/ml, $p<0.05$). Hypothalamic neuropeptide Y (NPY) mRNA expression, however, was unchanged, even when combined with pair-feeding, suggesting that increased food intake with high dose RSG treatment is not mediated by NPY. **Conclusion**: Combination of food restriction with RSG improves insulin sensitivity more than RSG alone in DIO rats, and this effect is independent of a fall in FFAs.

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DARGLITAZONE-INDUCED BODY WEIGHT GAIN IN THE OBESE ZUCKER RAT: ROLES OF FOOD INTAKE AND MELANOCORTIN-4 SATIETY RECEPTOR.

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Thiazolidinediones, effective in the treatment of type 2 diabetes, have significant body weight gain effects in insulin-resistant animal models. Aims: To determine involvement of changes in food intake versus energy expenditure in darglitazone-induced body weight gain in obese Zucker rats. To elucidate the role of satiety signalling via the central melanocortin-4 receptor (MC4-R) in this effect. Materials and Methods: Male obese Zucker rats, treated with darglitazone (1.3μmol/kg.day), were either freely fed or food restricted (by pair feeding to freely fed obese controls). Food intake, body weight and body composition were monitored. After a 3 week treatment period, MC4-R levels in brain slices were assessed by quantitative autoradiography using 70pM [^{125}I]NDP-MSH. Results: At the end of 3 weeks, darglitazone treatment markedly lowered plasma insulin (by 86%, P<0.05) and triglycerides (by 82%, P<0.05) from pretreatment values. Comparable reductions were seen in the food-restricted treated animals. There were no differences in plasma leptin levels, between the three groups of these hyperleptinaemic animals. Darglitazone-treatment increased food intake by 31% (P<0.05) mainly by altering the diurnal feeding pattern and produced significant increase in body weight gain (by 77%, P<0.05) due to a selective increase in fat body mass. In food-restricted animals, there was no darglitazone-induced increase in weight gain or fat mass, demonstrating that altered metabolic efficiency does not significantly contribute to the weight gain effect. MC4-R levels in the hypothalamic arcuate and ventromedial nuclei were significantly decreased in the freely-fed, darglitazone-treated group (by 20% in both areas as compared to controls, P<0.05). The opposite changes were observed in the same brain regions of food-restricted darglitazone-treated group (20% and 37% increase respectively, P<0.05). Conclusions: The marked body weight gain effect of darglitazone in obese Zucker rats can be totally accounted for by the increased food intake. In these rats with an impaired leptin signalling system, the observed large regionally selective changes in MC4 receptor levels were most likely mediated via leptin-independent mechanisms.

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EFFECT OF DEHYDROEPIANDROSTERONE ON GLUCOSE METABOLISM IN LIVER AND MUSCLE: COMPARISON WITH TROGLITAZONE

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Aims: It has been reported that dehydroepiandrosterone (DHEA) improved hyperglycemia in diabetic C57BL/KsJ-db/db mice. Troglitazone is a hypoglycemic agent that has been recently introduced into clinical medicine to treat diabetic patients that are insulin resistant. Aim is to elucidate the mechanism of the respective hypoglycemic effect of DHEA and troglitazone in db/db mice.

Materials and methods: We administered DHEA, troglitazone and androstenedione, respectively to db/db and db/+m mice. We measured photometrically hexokinase+glucokinase (HK+GK), phosphofructokinase (PFK), pyruvate kinase (PK), glucose-6-phosphatase (G6Pase), fructose-1,6-bisphosphatase (FBPase), and phosphoenolpyruvate carboxykinase (PEPCK) in liver and muscle in db/db mice and db/+m mice.

Results: Despite hyperinsulinemia, hepatic G6Pase and FBPase activities are higher in db/db mice than in db/+m. Administration of DHEA and that of troglitazone significantly decreased blood glucose in db/db mice and hepatic G6Pase and FBPase activities both in db/db and db/+m mice. Hepatic G6Pase and FBPase activities showed a linear relationship with blood glucose in all the groups of mice. Androstenedione, a DHEA metabolite, barely affected either of these enzyme activities or blood glucose in db/db mice.

Conclusions: These data suggest that the activities of G6Pase and FBPase are closely related to blood glucose levels and these actions of DHEA are presumed to be caused by DHEA itself.

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THE THIAZOLIDINEDIONE, PIOGLITAZONE, INCREASES IRS-2 GENE AND PROTEIN EXPRESSION IN 3T3-L1 CELLS.

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The PPAR agonist, pioglitazone, is a powerful insulin sensitizer in various animal models of insulin resistance and diabetes. However, the mechanisms for the insulin sensitizing effects of these agents are unclear.
Aims: To examine the effects of pioglitazone on upstream proteins related to insulin signaling and action.
Methods: Differentiated 3T3-L1 cells were exposed to pioglitazone (10^{-6} and 10^{-5} M) in the absence or presence of insulin for 24 and 48 hrs. Gene and protein expression of several intracellular signaling molecules were examined.
Results: The most striking effect of pioglitazone was seen on IRS-2 mRNA and protein expression which were increased 3-4-fold. This finding was seen both in the absence and presence of insulin in the culture medium. However, the gene and protein expression of several other signaling proteins, including IRS-1, were unchanged. The increased IRS-2 expression was also accompanied by an increased binding of PI3-kinase (p85 subunit) to this docking protein.
Conclusions: Pioglitazone increases the gene and protein expression of IRS-2 in 3T3-L1 cells. This may be an important reason for the insulin sensitizing effect of this thiazolidinedione.

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IDENTIFICATION OF NATURAL LIGANDS FOR THE α AND γ ISOFORMS OF HUMAN PEROXISOME PROLIFERATORS-ACTIVATED RECEPTOR

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Peroxisome proliferators-activated receptors (PPARs) α and γ are orphan receptors, which regulate the expression of genes involved in lipid metabolism. Several fatty acids and their metabolites were identified as natural ligands for human PPARγ. However, to date there was no direct evidence for ligand binding activity to human PPARα. We recently developed a novel thiazolidinedione (KRP-297), a ligand for both hPPARα (Kd, 228 nM) and hPPARγ (Kd, 326 nM). In the present study, we examined the ability of fatty acids and their metabolites to bind directly to hPPARα and hPPARγ using radiolabeled [^{3}H]KRP-297 in a competitive binding assay. **Results: 1)** The binding of 100 nM [^{3}H]KRP-297 to hPPARα was displaced by unlabeled KRP-297 (IC_{50}, 0.5 μM) and the fibrate Wy-14643 (IC_{50}, 5.4 μM). The [^{3}H]KRP-297 binding was displaced by palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2) with IC_{50} values of 4.3 μM, 1.7 μM, 0.9 μM, and 2.4 μM, respectively. Thus, in contrast to the findings in rodents, the saturated and unsaturated fatty acids were even more potent ligands of hPPARα than Wy-14643. The binding affinity of 8(S)-hydroxyeicosatetraenoic acid (HETE) for hPPARα (IC_{50}, 0.1 μM) was higher than that of its enantiomer, 8(R)-HETE (IC_{50}, 1.3 μM). **2)** The binding of 100 nM [^{3}H]KRP-297 to hPPARγ was displaced (>50%) by the unsaturated fatty acids (C18:1, C18:3, and C22:6) of 10 μM. The saturated fatty acids (C12-C18) and the unsaturated fatty acids (C18:2 and C20:4) were weaker ligands of hPPARγ. **3)** Moreover, we identified that a novel class of fatty acid metabolites was potent ligands of hPPARα and hPPARγ. **Conclusion:** Using a versatile tool for identification of ligands for hPPARα and hPPARγ, we demonstrated that naturally occurring fatty acids and their metabolites are ligands of these receptors and may function as critical regulators of lipid homeostasis.

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THE ASSOCIATION OF HIGH FASTING IMMUNOREACTIVE INSULIN LEVELS AND CARDIOVASCULAR RISK FACTORS: A POPULATION STUDY.

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Aims: To establish the distribution of fasting immunoreactive insulin (IRI) levels in our population and its association with cardiovascular risk factors, and also with the "impaired fasting glucose" (ADA 1997) and "impaired glucose tolerance" categories.
Methods: In a representative council of our area, 683 subjects over 30 years old were chosen in a random stratified sampling of the population. They had an standard OGTT performed in the fasting state (excluding those who had been previously diagnosed as having diabetes mellitus or had fasting glucose > 7.8 mM/L). Height, weight, waist and hip perimeters and blood pressure were measured; fasting lipid profile (triglycerides, total and HDL cholesterol) and fasting IRI were determined.
Results: The fasting IRI in our population was 47.1 ± 38.3 pmol/L (mean + s.d.) The range was 1.4 – 429.2 and the median 37.9. The distribution did not fit the normal ($p<0.001$; Kolmogorov-Smirnoff test). Being in the highest IRI quartile (over 57.2 pmol/L) was significantly associated with the presence of hypertension (30.9% of the hypertensive subjects, $p=0.017$; chi-square), specifically with diastolic hypertension (46.7%; $p<0.001$); with hipertriglyceridemia (43.3%, $p<0.001$) but not with hypercholesterolemia (25.7%; $p=0.53$); with obesity (31.6%; $p=0.015$) and with central obesity (44.4%; $p<0.001$), but not with male sex (23.4%; $p=0.138$) nor age. The IFG category was also associated with the highest fasting IRI quartile (39.3%; $p=0.009$), but not the IGT category (26.5%; $p=0.102$)
Conclusions: We conclude that hyperinsulinemia is associated in our population with hypertension (specially with diastolic hypertension), with dislipidemia (specially with hypertriglyceridemia) and with obesity (specially with central obesity). Impaired fasting glucose, but not impaired glucose tolerance is also associated with hyperinsulinemia. A causative effect of insulin resistance on this risk factor clustering is suspected but remains unproven.

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Effects of Hormone Replacement Therapy on Insulin Resistance in Postmenopausal Women

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Aims: In the present study our aim was to investigate the effect of hormone replacement therapy (HRT) on insulin resistance in postmenopausal women.
Materials and Methods: At the start, 39 postmenopausal women were enrolled in the study. The patients were not included if they had diabetes mellitus, impaired glucose tolerance, obesity (body mass index >30 kg/m^2), systemic diseases, or history of previous hormone replacement therapy. Fifteen patients out of 39 were found to have insulin resistance and placed on hormone replacement therapy, consisting of conjugated equine oestrogen (0.625 mg/day) and medroxyprogesterone acetate (10 mg/day), for 3 months. Euglycaemic hyperinsulinemic clamp technique was used to determine insulin resistance before and after HRT. In this tecnique, mean M value was used as a main objective.
Results: The mean age was 50.6±6.4 (ranged between 37-59) years. The mean pretreatment levels of M value, insulin, C-peptide, total cholesterol (TC), triglyceride (TG), high density-lipoprotein cholesterol (HDL-C) and light density-lipoprotein cholesterol (LDL-C) were found as 3.3±0.6 mg/kg/min, 39±10.1 μIU/dl, 3.0±1.1 ng/ml, 230±43 mg/dl, 152±65 mg/dl, 41.8±6.1 mg/dl and 188±6.4 mg/dl, respectively, while the same values after 3 months' hormone replacement therapy were as follows; 4,54±0.9 mg/kg/min, 26,6±10.1 μIU/dl, 3,57±0.6 ng/ml, 209±27 mg/dl, 132±37.9 mg/dl, 48.4±3.9 mg/dl and 161±8.2 mg/dl, respectively. With the hormone replacement therapy, meaningful improvements occurred in the levels of insulin, LDL-C, HDL-C and the M values. However, there were no statistically significant changes in the levels of TG, C-peptide and basal glycaemia. The M value, which was the main objective of our study, was increased 28% by HRT ($p<0.001$). Also, there were 2.9% decrease in LDL-C ($p<0.044$), 9.1% in TC ($p<0.016$), 33% in serum insulin level ($p<0.022$) and 17% increase in HDL-C ($p<0.009$) levels while there were no statistically significant changes in the levels of C-peptide and TG ($p>0.05$).
Conclusions: Our study shows that hormone replacement therapy has useful effects on insulin resistance. This therapy may also improve cardiovascular risk factors in addition to a number of other useful effects.

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INSULIN SECRETION AND RESISTANCE IN NORMAL GLUCOSE TOLERANT OFFSPRING OF TYPE 2 DIABETIC PATIENTS

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AIMS: Type 2 diabetes mellitus is characterized by decreased insulin secretion and decreased tissue sensitivity to insulin. However, it has been unclear which of these abnormalities precedes the other in the different ethnic groups. We studied to identify early metabolic abnormalities in the pathogenesis of diabetes in normal glucose tolerant offspring (n=151) of diabetic patients and control subjects (n=89) with negative family history-matched for age, sex and body mass index. MATERİALS AND METHODS: All the subjects underwent a 75 g oral glucose tolerance test. Insulin resistance was determined by homoestasis model assessment (HOMA) and fasting insulin levels. The abnormalities in insulin secretion were defined by the ratio of the 30-min change in insulin to the 30-min change in glucose ($\Delta I_{30}/\Delta G_{30}$). RESULTS: No significant difference were found in fasting and post-challenge glucose levels (120 min) in both groups. Basal serum insulin level and HOMA index in offspring of diabetic patients with normal glucose tolerance tended to be slightly higher than in matched controls (116.6±6.8 vs 88.7±8.2 pmol/L, P=0.08; 4.7±0.9 vs 3.5±0.4 mmol.mU^{-1}.l^{-2}, P=0.06 respectively). $\Delta I_{30}/\Delta G_{30}$ in offspring of diabetic patients was significantly lower than in controls (137.5±11.6 vs. 176.3±48.4 pmol.mmol^{-1}, P=0.030). However, HOMA insulin resistance index and fasting serum insulin were correlated with insulin secretion (r=0.4904 P=0.000 and r=0.6730 P=0.000, respectively). CONCLUSIONS: These data suggest the early decreased insulin secretion in response to an oral glucose challenge rather than insulin resistance may be the important factor in the future development of diabetes in Turkish population at high risk of diabetes.

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INSULIN SENSITIVITY AND INSULIN SECRETION IN PATIENTS WITH MITOCHONDRIAL GENE MUTATION AT POSITION 3243

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Aims: The mitochondrial DNA mutation at position 3243 (3243-mutation) is not only associated with MELAS (Mitochondrial Encephalomyopathy, Lactic Acidosis and Strokes) but also with diabetes mellitus. The aim of the present investigation was to study insulin sensitivity (SI), insulin secretion ($AIR_{Glucose}$) and glucose effectiveness (Sg) in patients with the 3243-mutation. **Patients and Methods:** 6 patients (1 female, 5 male) of a large pedigree and 3 siblings (2 female, 1 male) of another family in whom the 3243-mutation had been detected (age 33 years [19-44], BMI 23.3 kg/m^2 [15.9-33.1]) and 9 matched non-related control subjects underwent a modified intravenous glucose tolerance test (Bergman's minimal model: 0.33g/kg glucose, tolbutamide protocol). **Results:** All patients showed normal glucose tolerance. There was no difference between patients and controls for insulin sensitivity (SI: 9.33 ± 2.96 vs 8.39 ± 1.74 $*10^{-4}$ min^{-1}/mU/l), glucose effectiveness (Sg: 2.7 ± 0.7 vs 1.9 ± 0.3 $*$ 10^{-2} min^{-1}) or first-phase insulin response ($AIR_{Glucose}$: 322.3 ± 140.1 vs 245.1 ± 60.9 $*10^{-12}$ mol/l; mean ± SEM). However, when looking at the individual results, there were 4 closely related members of the large pedigree with very poor insulin sensitivity (SI < 3.45$*10^{-4}$ min^{-1}/mU/l). The other 2 patients of this pedigree were more distantly related and extremely insulin sensitive (SI > 16.50$*10^{-4}$ min^{-1}/mU/l). The siblings of the other family showed normal or even very good insulin sensitivity. In one patient of the large pedigree islet cell antibodies (ICA) and glutamic acid decarboxylase antibodies (GADA) were detected. **Conclusions:** Insulin resistance solely does not cause impaired glucose tolerance in patients with the 3243 mutation. The differences in insulin sensitivity in members of a large pedigree may lead to the conclusion that in the pathogenesis of mitochondrial diabetes further genes are involved. In the presence of ICA and GADA, an underlying autoimmune process has to be considered.

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ACQUIRED GENERALIZED LIPOATROPHY (AGL) AND SEVERE INSULIN RESISTANCE (IR): EVIDENCE FOR MUSCULAR GLYCEROL RELEASE (MGR) AND INSULIN-INDUCED SUPPRESSION OF LIPOLYSIS (ISL)

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Aims: Increased muscular lipid content has been shown to be associated with insulin resistance. In situ microdialysis (MD) revealed that muscular lipolysis is under hormonal control, i.e. suppression of glycerol release by insulin (I). However, currently it is not known, whether this glycerol originates from intra- (IMCL) or extramyocellular lipids (EMCL). To determine the source of glycerol assessed by muscular MD, we investigated a patient with AGL, which is a rare disorder of unknown etiology associated with almost complete absence of subcutaneous adipose tissue and severe IR.

Methods and results: The male patient, age 30, BMI 16.1kg/m², fasting insulin (I) 47 mU/ml, required 160 U/day of exogenous I (I-Lispro and Semilente) in addition to 1.700 mg metformin/day. We determined (i) the EMCL and IMCL-pool by magnetic resonance spectroscopy (MRS), (ii) the MGR by MD in tibialis ant.muscle (TAM) and gastrocnemius muscle (GM) during fasting and a three step euglycemic hyperinsulinemic glucose clamp (GC) (GC-I=0.1, II=0.25 and III=1.0mU/ml*kg*min) to quantify ISL. MRS-studies revealed a total lack of EMCL in all muscles investigated and a reduced pool of IMCL in all muscles investigated when compared to controls. Microdialysis revealed basal release of glycerol (0,23mg/dL) which remained unchanged during GC-I, but was suppressed by GC-II and GC-III (0,13/0,1mg/dl). Insulin sensitivity as measured by MCR was only 1,35 and 2,6ml/kg*min (at GC-II and GC-III), indicating severe insulin resistance.

Conclusion: In a patient with AGL, muscular glycerol release can be followed despite a complete lack of EMCL, as shown by the MRS. These data strongly suggest, that the glycerol measured in muscular MD most likely originates from IMCL.

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MICROVASCULAR ANGINA (CARDIOLOGICAL SYNDROME X) IS NOT ASSOCIATED WITH HYPERINSULINEMIA OR INSULIN.RESISTANCE

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Aims: Microvascular angina has been found to be associated with insulin resistance. However, many factors known to affect insulin sensitivity were not excluded in patient selection. We aimed to evaluate wheather microvascular angina is per se associated to insulin resistance. **Materials and Methods**: We performed a Frequently Sampled Intravenous Glucose Tolerance Test (0.33 g/kg b. w.) in 10 normal weight and normotensive patients with microvascular angina, with normal glucose tolerance and normal plasma lipids. Ten healthy subjects, comparable for age, sex, body mass index, blood pressure and plasma lipids, were used as control group. **Results**: Fasting serum glucose (4.49±0.2 SEM vs 4.52±0.13 mmol/l, p=0.9), insulin (39.46±3.68 SEM vs 47.12±4.6 pmol/l, p=0.21) and C-peptide (0.56±0.05 SEM vs 0.53±0.05 nmol/l, p=0.68) values, as well as estimated parameters of insulin secretion (area under the curve of C-peptide: 211.84±26.48 SEM vs 194.25±19.99 nmol/l/240 min, p=0.6) and hepatic insulin extraction (76.2±4.1 SEM vs 73.92±5.87 %, p=0.75) were similar in the two groups. Insulin sensitivity was also similar in the patients and control subjects (7.02±1.28 SEM vs 8.17±1.28 . 10^{-4}. min^{-1}/(μU/ml), p=0.53). **Conclusions**: Microvascular angina per se is not associated with hyperinsulinemia or insulin resistance when other confounding factors are excluded in patient selection.

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GLUCOSE AND LIPID METABOLISM IN IDIOPATHIC NONALCOHOLIC STEATOHEPATITIS.

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Aim: Idiopathic Nonalcoholic Steatohepatitis (NASH) is a hitherto poorly understood liver disease, histologically mimicking alcoholic steatohepatitis, that may progress to cirrhosis in a variable percentage of cases (8-40 %). We wanted to investigate the association of NASH with abnormalities of daily alimentary intake, of postprandial lipid metabolism and of glucose metabolism. **Materials and Methods:** Eleven histologically confirmed cases of NASH (age 41±12, BMI 26±2) and ten matched controls underwent a complete diethological study of alimentary habits, an oral glucose tolerance test (oGTT), a frequently sampled intravenous glucose tolerance test (whose data were analyzed with the MINIMAL MODEL method) and a 10-hour standard oral fat tolerance test with measurement of plasma total triglyceride (TG), of VLDL-TG and of TG content in VLDL subfractions. **Results:** Diethological analysis revealed a hypercaloric intake of the patients (+360 Kcal/die over the ideal), with differences affecting especially "simple" carbohydrates and saturated fatty acids. Cases showed significantly reduced insulin-sensitivity (insulin sensitivity index, SI: 3.66±2.86 vs 8.52±3.81 $min^{-1}/(\mu U/ml)$, P<0.05) and elevated insulinemia (total insulin area; TIA: 8.32±5.43 vs 3.35±1.28 mU/ml/240', P<0.05); three of them were also glucose intolerant on the oGTT. On oral fat tolerance test cases showed an increase in absolute TG content in VLDL subfraction A at +4h (0.44±0.32 mg_{Tot} vs 0.09±0.06 mg_{Tot}, P=0.03) and in percentual TG content in VLDL subfraction D at +0h (57±7% vs 49±5.7%, P=0.05) and +2h (40±6% vs 28±3%, P=0.02). **Conclusions:** We conclude that NASH is associated with a reduced insulin-sensitivity and an abnormal distribution of TG among endogenous triglyceride-rich lipoproteins, whose underlying mechanism and implication are still to be elucidated.

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RELATIONSHIP BETWEEN INSULIN RESISTANCE AND PROGRESSION OF NEPHROPATHY IN NIDDM SUBJECTS

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Aims: To assess the relationship between insulin resistance and progression of nephropathy in NIDDM subjects, we measured a glucose infusion rate (GIR), and evaluated baseline clinical characteristics and microvascular complications.

Subjects and Methods: NIDDM subjects were divided into four groups: no complications, Group 1 (n=14); normoalbuminuria with neuropathy, Group 2 (n=21); microalbuminuria, Group 3 (n=12); macroalbuminuria, Group 4 (n=7). CV R-R and 24-hr urinary C-peptide excretions were measured in all subjects. GIR indices for insulin resistance were calculated from euglycemic clamp studies. Comparison of mean values were performed by Kruskal-Wallis's rank test among four groups. Relationships between degree of nephropathy and other variates were evaluated by multiple regression analysis. The normal range for GIR was defined as within 2SD of mean value from control subjects. Diabetic subjects were divided into two groups for discriminating reduced GIR; subjects with normal and decreased GIRs, relationship between GIR and clinical characteristics were examined by logistic regression analysis.

Results: There was no difference in age, BMI, or HbA1c among four groups. Durations of diabetes were longer, mean blood pressure (MBP) levels were higher, and GIR levels were lower in Group 4 than in Group 1. Multiple regression analysis revealed that the progression of nephropathy was strongly related to duration of diabetes, urinary C-peptide excretion, and MBP. Furthermore, based on logistic regression analysis, duration of diabetes, HbA_{1c}, HDL-cholesterol, FFA, and albumin excretion rate (AER) were useful for discriminating a reduced GIR.

Conclusions: Our data suggest that urinary AER and duration of diabetes as well as glycemic control, HDL-cholesterol, and FFA were asscociated with the degree of insulin resistance, and that advanced diabetic nephropathy was one of factors to reduce GIR.

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INCREASED INTERSTITIAL LACTATE CONCENTRATION IN PERIPHERAL ADIPOSE TISSUE IN OFFSPRING OF TYPE 2 DIABETES PATIENTS

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Aims: To study the interstitial lactate concentration in peripheral adipose tissue in healthy first-degree relatives of type 2 diabetes patients.

Materials and methods: Seven diabetes relatives (Rel) and 7 pair-wise matched controls (Con) were studied after an overnight fast (Gender: 4M/3F vs 4 M/3F, Age 35 ± 1 vs 34 ± 1 yrs (Mean ± SE), BMI: 23,5 ± 0.7 vs 23,1 ± 0.5 kg/m^2). All subjects underwent a euglycemic hyperinsulinemic clamp, abdominal subcutaneous microdialysis, ^{133}Xenon clearance and subcutaneous needle biopsies.

Results: In the fasting state, there was no difference in arterial plasma lactate (Rel: 0.78 ± 0.09 vs Con: 0.58 ± 0.01 mmol/l, n.s.) but the interstitial lactate concentration was increased in diabetes relatives (Rel: 1.36 ± 0.23 vs Con: 0.96 ± 0.08 mmol/l, $p < 0.05$). Moreover, adipose tissue blood flow was slightly lower in the relatives (Rel: 2.9 ± 0.4 vs 4.0 ± 0.6 ml/100 g/min, n.s). During the insulin clamp (B-glucose: 5.1 ± 0.0 vs 5.1 ± 0.1 mmol/l, n.s., S-insulin 102 ± 6 vs 85 ± 4 mU/l, $p < 0.05$) the relatives tended to have an impaired glucose disposal (Insulin sensitivity index: 12.61 ± 1.74 vs 16.23 ± 0.88 100 x mg x l/kg lean body mass/min/mU, $p = 0.063$). Interestingly, they also had larger subcutaneous fat cells (98 ± 3 vs 82 ± 3 µm, $p < 0.02$) and, in addition, the fasting interstitial lactate concentration correlated with the fat cell size ($p < 0.05$, $r^2 = 0.547$).

Conclusions: Our data suggest that first degree relatives of type 2 diabetes patients have an increased interstitial abdominal subcutaneous lactate concentration indicative of an enhanced non-oxidative glucose metabolism.

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SURGICAL THERAPY IMPROVES INSULIN ACTION IN PRIMARY HYPERALDOSTERONISM

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Aims: Primary hyperaldosteronism was found to be associated with impaired insulin action. We decided to evaluate the effect of surgical and pharmacological treatment of primary hyperaldosteronism (PH) on insulin action. **Patients and methods:** Nine patients with PH confirmed by the presence of arterial hypertension, low serum potassium concentration, high plasma aldosterone and low renin levels as well as by the findings on CT scans were evaluated in this study. All had normal glucose tolerance. Aldosterone producing adenoma was later removed in five patients (Group A) whereas spirolactone was used in the remaining four with idiopathic hyperaldosteronism (Group B). Two control groups of corresponding body mass index (C_1 n=5, and C_2, n=4)) were used for comparison. Hyperinsulinemic euglycemic clamps using insulin infusion rate 1 mU/kg/min were performed before and 6 months after surgical removal of an adenoma or after spirolactone treatment. Additionaly, insulin receptor characteristics were evaluated in all patients. **Results:** Biochemical variables from the clamps are shown in Table 1.

Variable	Group A		C_1	Group B		C_2
	Before	After		Before	After	
M (µmol/kg/min)	19.4±3.1$^{\wedge}$	39.4±10.5	37.5±3.0	21.7±5.9	18.5±5.7	27.9±8.2
M/I (µmol/kg/min/mU/l	30.2±5.9$^{\wedge}$	51.4±12.2	48.9±5.0	24.5±7.3$^{\wedge}$	18.7±7.6	33.6±9.6
MCR_G (ml/kg/min)	3.9±0.7$^{\gamma}$	7.4±2.1	8.6±1.1	4.6±2.1$^{\wedge}$	3.5±1.1$^{\wedge}$	6.2±2.0

Statistical differences as compared to controls: $^{\wedge}p<0.01$, $^{\gamma}p<0.001$

Significantly decreased glucose disposal rate (M), insulin sensitivity index (M/I) and metabolic clearance rate of glucose (MCR_G) were found in patients with PH as compared to controls. The improvement of the above variables was observed only after surgical removal of an adenoma whereas no effect on insulin action was found after spirolactone treatment. The insulin receptor characteristics were not significantly influenced. **Conclusion:** The insulin resistance is present in patients with PH but it may be significantly improved only in surgically treated patients.

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Insulin Sensitivity Measurements

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A METHOD FOR ASSESSING INSULIN SENSITIVITY FROM THE OGTT

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Introduction and Aim. The OGTT is used for diagnosis in diabetes, but does not yield an index of insulin sensitivity (IS). We have developed a formula to determine IS from the OGTT, based on a glucose-insulin model. **Methods.** Glucose and insulin from a standard 75g OGTT at 0, 2, and 3 hrs are used to predict glucose clearance measured independently during a 120 $mUmin^{-1}m^{-2}$ euglycemic clamp. The formula has 6 constants, optimized by fitting OGTT-predicted glucose clearance (OGC) to clamp clearance (GC), calculated as the ratio of glucose infusion to concentration during the last hour. 91 subjects (Controls (NGT): n=15, BMI<25; Obese: n=38, BMI>25; NIDDM: n=38) randomly underwent the OGTT and the clamp. **Results.** OGC and GC were well correlated in the whole group (R=0.78, p<0.0001) and in the subgroups (R=0.49 to 0.73, p<0.02 or less). OGC and GC equivalently detected IS differences between groups (for OGC, NGT vs Obese: 440±16 vs 362±11 ml $min^{-1}m^{-2}$, p<0.001; NGT vs NIDDM: 440±16 vs 239±7, p<0.0001; Obese vs NIDDM: 362±11 vs 239±7, p<0.0001, similar results for GC). As expected, in nondiabetic subjects GC was inversely correlated with BMI and fasting plasma insulin (FPI) (R=-0.65 to -0.67, p<0.0001, after bi-log transformation). Equivalent results were found for OGC (R=-0.64 to -0.69, p<0.0001). To validate the method, OGC was tested on a separate group of 13 intolerant subjects (IGT), who underwent paired OGTT and clamp. OGC correlated significantly with GC also in this group (R=0.65, p<0.02). In IGT too, OGC correlated inversely with BMI and FPI (R=-0.78 to -0.90, p<0.002 or less). The relationship between IS and insulin secretion was also examined. A β-cell sensitivity index (βSI) was calculated as the ratio of insulin to glucose increments during the OGTT. The expected inverse correlation between OGC and βSI was found in NGT, Obese, and IGT subjects (R=-0.66 to -0.84, p<0.001 or less, bi-log). **Conclusion.** This OGTT-based method requiring only 3 blood samples provides an index of IS in good agreement with the clamp and reproduces known facts concerning IS. The method has therefore potential use in clinical investigation.

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CORRELATION OF FASTING INSULIN AND HOMA MODEL WITH INSULIN SENSITIVITY INDEX - THE KANWU STUDY

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Aims: Different methods have been developed to evaluate insulin sensitivity in population studies. We examined the correlations of insulin sensitivity index with fasting insulin and HOMA index. **Materials**: The KANWU study is an international multicentre study established to examine the effect of dietary fat quality on insulin sensitivity in middle-aged non-diabetic subjects (n=162, 86 men, 76 women, age 48.4±7.6 years for men and 48.8±8.0 for women, mean ±SD). **Methods**: All the measurements we carried out in an overnight fasted condition before any dietary intervention. Insulin sensitivity index (Si) was based on the frequently sampled intravenous glucose tolerance test, fasting insulin (FI) was obtained in the beginning of a 2-h glucose tolerance test (performed to exclude diabetic subjects) and HOMA index was calculated. **Results**: The correlation between Si and FI (r=-0.52, p=0.001) was similar to that between Si and HOMA (r=-0.55, p=0.001). Both of these correlations were slightly stronger in men than in women (r=-0.63 and r=-0.64 and r=-0.49 and r=-0.52, for men and women, respectively). The degree of obesity had no significant impact on these correlations. Si declined along with an elevation of FI as analysed by insulin tertiles; 6.41±3.33, 3.98±1.74, 2.94±1.87 ($x10^{-5}$ min^{-1} (pmol/l), n=156, p=0.001 for trend. **Conclusions**: FI can be used as a surrogate index for the evaluation of insulin sensitivity. HOMA does not seem to offer any additional benefit beyond FI in a non-diabetic population.

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COMPARISON OF DIFFERENT METHODS IN ASSESSMENT OF INSULIN RESISTANCE IN WOMEN WITH POLYCYSTIC OVARIAN SYNDROME

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Aims: Hyperinsulinemia and peripheral insulin resistance (PIR) are recognised characteristics of polycystic ovarian syndrome (PCOS). In this study we compared the sensitivity of single-sample basal insulin, mean of three consecutive samples of basal insulin, HOMA and CIGMA in patients with PCOS (n: 25, age: 29.4 ± 1.6 yrs) and healthy control subjects (n: 11, age: 25.8 ± 2 yrs). **Materials and Methods:** The diagnosis of PCOS made by specific findings on ultrasonography, hirsutism, oligo- or amenorrhea and increased androgen levels. None of the patients had diabetes and impaired glucose tolerance as defined by a standart OGTT. Basal insulin levels assessed by mean of three consecutive samples take in at least 10 minutes recumbeat with 5 minute intervals after an overnight fast. Corresponding three samples for blood glucose were obtained for HOMA test and then a CIGMA test was performed. **Results:** Both single-sample basal insulin and mean of three samples for basal insulin as well as R values for HOMA and CIGMA were significantly higher in PCOS group than in controls (single sample basal insulin: 12.1 ± 6.8 vs 5.1 ± 2.9 mU/ml, p<0.001; mean of three samples basal insulin: 12.8 ± 6.9 vs 5.1 ± 1.8 mU/ml, p<0.000; HOMA-R: 2.4 ± 1.3 vs 0.8±0.3 mU/ml, p<0.000, CIGMA-R: 2.3±1.6 vs 0.6±0.2 mU/ml, p<0.000). Single sample basal insulin was found to be highly correlated to other three methods. (Mean of three samples: r=0.93, p<0.000; HOMA: r=0.90, p<0.000; CIGMA: r=0.41, p<0.01). **Conclusions:** The results suggested that the validity of single sample basal insulin is comparable to relatively more complex and invasive technics. We conclude that single sample basal insulin is a reliable method to estimate PIR in PCOS. Since it is simple, straight forward and cheap, it can be used in large scale studies.

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ASSESSMENT OF INSULIN-SENSITIVITY AND BETA-CELL FUNCTION FROM AN ORAL GLUCOSE TOLERANCE TEST.

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Aims: Can measurements of glucose and insulin during an OGTT predict insulin sensitivity and β–cell function in individuals with normal glucose tolerance (NGT). **Material and Methods:** NGT offspring (n=219) and NGT living spouses (n=29) (55.8 % females; mean age 41.2, SD=11.2 years; mean BMI 25.9, SD=4.3 kg / m^2) from 60 Danish Caucasian families with one parent with Type 2 diabetes underwent an OGTT (75g glucose; 18 samples during 240 min) and an IVGTT (0.3 g glucose / kg; 3 mg tolbutamide / kg injected at 20 min; 33 samples during 180 min). Bergman's minimal model was used to calculate insulin sensitivity (S_I) and the Acute Insulin Response (AIR_{0-8}). Multiple linear regression was used to derive predictive equations of log(S_I) and log(AIR) using OGTT glucose and insulin, BMI and gender from a random 75% sample of the data set. The predictions were validated by applying the derived models to the remaining 25% of the data set. **Results:** OGTT-S_I: Optimal model (glucose and insulin at 0, 30, 60, 105, 180, 240 min; BMI and gender): R^2 = 0.78 (SD = 0.31). Simple model (glucose and insulin at 0, 60 and 120 min; BMI and gender): R^2 = 0.71 (SD = 0.32). OGTT-AIR: Optimal model (glucose and insulin at 0, 10, 50, 120 min, BMI and gender): R^2 = 0.54 (SD = 0.38). Simple model (glucose and insulin at 0, 60 and 120 min, BMI and gender): R^2 = 0.48 (SD = 0.40). Validation: OGTT-S_I: Optimal model: R^2 = 0.77 (SD = 0.42). Simple model: R^2 = 0.78 (SD = 0.38). OGTT-AIR: Optimal model: R^2 = 0.59 (SD = 0.49). Simple model: R^2 = 0.62 (SD = 0.48). **Conclusions:** A standard OGTT with measurements of glucose and insulin at 0, 60 and 120 min plus BMI and gender makes it possible to predict estimates of S_I and AIR which are highly correlated to IVGTT-derived S_I and AIR. The assessment of S_I and AIR from an OGTT can be applied in large-scale epidemiological studies of subjects with normal glucose tolerance.

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COMPARATIVE EVALUATION OF SIMPLE INDICES BASED ON FASTING PLASMA INSULIN TO ASSESS INSULIN SENSITIVITY

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Aims : To compare various simple indices of insulin action based on fasting plasma insulin levels with the minimal model-derived insulin sensitivity index S_I in non-diabetic subjects with a large range of BMI. **Materials and Methods :** 108 non-diabetic subjects (29 males and 79 females with a BMI ranging from 17.5 to 47.8 kg/m², aged 33.9 ± 12.0 SD years) were submitted to an intravenous glucose tolerance test (0.3 g glucose /kg body weight). The insulin sensitivity index (S_I) obtained by the minimal model approach was compared to various indices based on basal plasma insulin (+ glucose) levels: fasting insulin levels (FI), fasting glucose to insulin ratio (FGIR), fasting insulin resistance index (FIRI : FI x glucose/ 25) and insulin sensitivity obtained by the HOMA model (%S). **Results :** As expected and because of the heterogeneity of the population, a large distribution of the various indices was observed : FI varied from 9 to 483 pmol/L, FGIR from 0.07 to 3.78 mmol.mU, FIRI from 0.19 to 12.52 mmol.mU.L^{-2}, %S from 7.5 to 428.1 and S_I from 0.39 to 54.75 10^{-5} min^{-1}.pmol^{-1}.L. All indices were significantly correlated with S_I with $p < 0.05$. However, the correlation coefficients were rather weak (FI : r = - 0.268; FGIR : r = 0.289; FIRI : r = - 0.248; %S : r = 0.288) and no index appeared to be more strongly related to S_I than simply fasting plasma insulin levels. **Conclusions :** In a non-diabetic population comprising individuals with markedly different values of BMI, the HOMA model does not provide an insulin sensitivity index which is better correlated to the minimal-model derived S_I index than fasting plasma insulin level, fasting glucose to insulin ratio or fasting insulin resistance index.

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RELATIONSHIP BETWEEN ENDOGENOUS GLUCOSE PRODUCTION AND INSULIN SENSITIVITY IN HUMANS

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Aims: Whether hepatic (as the fasting rate of endogenous glucose production (EGP)) and peripheral insulin sensitivity (as the insulin-mediated glucose uptake (M)) are related to one another in non-diabetic humans has not been determined. Materials and Methods: A primed-continuous infusion of [^{3}H]glucose (to measure fasting EGP) and a euglycaemic insulin clamp (7 pmol·min^{-1}·kg^{-1}, to measure M) were performed in 281 non-diabetic subjects (164 men and 117 women from the EGIR study) covering a wide range of age (18-85 yrs) and body mass index (15-55 kg·m^{-2}). Fasting post-hepatic insulin clearance (CRi, mostly reflecting hepatic insulin removal) was calculated from the ratio of exogenous insulin infusion to the steady-state plasma insulin concentration. Results: EGP ranged from 0.16 to 1.99 mmol/min, and was related to the lean body mass (r=0.54, p<0.0001) equally in men and women and more strongly than to any other index of body mass (body weight, surface area, BMI, fat mass, and percent fat mass). Furthermore, EGP was higher in subjects with a higher CRi (r=0.33, p<0.0001). After adjusting for these co-variates (as well as the fasting plasma insulin level) by multiple regression, EGP was positively related to age (partial r=0.24, p<0.0001) and negatively related to insulin sensitivity (partial r=0.16, p<0.01).

Conclusions: (a) In the fasting state, glucose production (which equals glucose utilisation) is set by the glucose requirement of lean body tissues, with no effect of adiposity per se; (b) The faster insulin clearance (mostly hepatic) associated with higher EGP suggests that weaker insulin inhibition of glucose release may be linked with shorter insulin residence times on liver receptors; (c) Subjects with peripheral insulin resistance have intrinsically more severe hepatic insulin resistance, and (d) Independently of other factors, EGP increases with age, suggesting a role for this physiological change in age-related loss of glucose tolerance.

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THE INTRAVENOUS PRANDIAL GLUCOSE TOLERANCE TEST (IVPGTT) MINIMAL MODEL FOR MEASURING GLUCOSE EFFECTIVENESS AND INSULIN SENSITIVITY.

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Aims: The IVGTT though widely employed for measuring glucose effectiveness and insulin sensitivity with the minimal model method, produces unphysiological profiles of glucose and insulin which exacerbates errors due to assumption of single-pool glucose kinetics. We develop here a more physiological test (IVPGTT) using meal-like glucose and insulin profiles which make the single-pool assumption less critical. **Materials and Methods:** Three protocols were performed in 10 healthy individuals. P1: somatostatin plus basal replacement of insulin, glucagon and GH plus glucose and labeled [6-^{3}H] glucose infused for 360 min so as to reproduce the rate of entry of a 50 gr. carbohydrate meal. P2: same as P1, with insulin also infused so as to mimic prandial insulin levels. IVPGTT: same as P2 with the omission of somatostatin, glucagon, and GH infusion. The data of P1, P2, IVPGTT were analyzed with the classic cold (CMM), hot (HMM) single-pool minimal models as well as the modified HMM (MHMM) which includes an inhibitory control of glucose on its own uptake. **Results:** Glucose Effectiveness: MHMM was only resolvable in P1, but glucose effectiveness on disposal $(SgV)_{hot}$ (where V is glucose distribution volume) of HMM and MHMM were, albeit different (1.60 and 0.96 ml·min^{-1}·kg^{-1} respectively), strongly correlated (r=0.85, p<0.01). HMM estimates of $(SgV)_{hot}$ in IVPGTT were highly correlated with those estimated in P1 (e.g. r=0.81, p<0.01). Also $(SgV)_{cold}$ were strongly correlated in P1 and IVPGTT (r=0.86, p<0.01). Insulin sensitivity on disposal $(SIV)_{hot}$ was identical in P2 and IVPGTT (0.12 ml·kg^{-1}·min^{-1} per mU·ml^{-1}) and highly correlated (r=0.904, p<0.001). $(SIV)_{cold}$ also was correlated, albeit less strongly (r=0.51, p>0.05). **Conclusions:** In conclusion, IVPGTT results in physiological changes in glucose and insulin which when interpreted with HMM yields an index of glucose effectiveness that is highly correlated with the "true" one and an index of insulin action that is virtually identical and highly correlated with the one calculated when endogenous insulin secretion is suppressed with somatostatin.

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Insulin Action and Cardiovascular System

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INSULIN INDUCED DECREASE IN LARGE ARTERY STIFFNESS IS IMPAIRED IN TYPE 1 DIABETES MELLITUS

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We have recently demonstrated that normal insulin action *in vivo* involves a decrease in stiffness of large arteries (a decrease in aortic pressure augmentation). This action of insulin is observed at physiological insulin concentrations within 0.5-1 h and precedes, by 1.5-2 h, changes in muscle blood flow. The magnitude of the decrease in arterial stiffness is associated with insulin resistance of glucose uptake in non-diabetic subjects. **Aims:** To determine whether the ability of insulin to decrease arterial stiffness is altered in type 1 diabetes. **Materials and methods:** Nine normal men (age 26±1 yrs, BMI 22±1 kg/m^2) and 9 uncomplicated type 1 diabetic men (age 28±2 yrs, BMI 24±1 kg/m^2) were studied under normoglycemic hyperinsulinemic [sequential 2 h insulin infusions of 1 (step I) and 2 (step II) mU/kg·min] conditions. Central aortic pressure waveforms were synthesized from those recorded in periphery using applanation tonometry on radial artery and a validated reverse transfer function to obtain central pressure waves every 30 min. This allowed determination of aortic augmentation (the pressure difference between the 1st and the 2nd systolic peaks) and the augmentation index (AgI, augmentation divided by pulse pressure), which is a measure of arterial stiffness. **Results:** Whole body glucose uptake was 44 (step I) and 37 (step II) % reduced ($p<0.001$) in the diabetic vs normal subjects. Augmentation averaged 0±1 and 2±1 mmHg in the normal and diabetic subjects basally (NS), and AgI -1.5±4.5 % and 4.0±3.7 % (NS), respectively. After 60 min of hyperinsulinemia, AgI had decreased significantly ($p<0.01$) to -9.5±4.8 % in the normal subjects but not in the diabetic patients (4.4±4.2 % at 60 min). A significant decrease was observed in the diabetic patients at 150 min (-1.2 ±4.1 %, $p<0.05$ vs basal). **Conclusions:** These data provide the first evidence of resistance of large arteries to insulin in type 1 diabetes.

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ASSOCIATION BETWEEN INSULIN RESISTANCE AT DIAGNOSIS OF DIABETES AND MACROVASCULAR COMPLICATIONS - THE UKPDS

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Insulin resistance is associated with an increased risk of cardiovascular disease in the general population, but few prospective studies in type 2 diabetes have evaluated this relationship. **Aims**: To estimate the association between insulin sensitivity (%S) and β cell function (%β) and subsequent myocardial infarction (MI), stroke, and lower extremity amputation (LEA) among subjects free of cardiovascular disease. **Methods**: 4623 of 5102 subjects recruited to the UKPDS had values for %S (median 22.4%) and %β (median 35.5%) estimated by Homeostasis Model Assessment measured 3 months following diagnosis of diabetes after a diet run-in. Up to 227 subjects with preexisting cardiovascular disease were excluded. %S and % β were log transformed and included in univariate or multivariate proportional hazards models with covariates traditionally associated with %S: age, ethnicity, sex, triglycerides, systolic blood pressure, and body mass. **Results**: No association between either %S or % β was observed for MI (n = 576) in univariate analyses over a median 10 years. In a multivariate model, a doubling of %β was associated with a 9% reduction in MI (95% confidence interval, [CI], 1%-16%, p=0.04). No associations were observed for %S or %β and stroke (n = 204). For LEA (n=51), a doubling of %S was associated with a 43% risk reduction (CI 16%-61% p = <0.005), a relationship not significant in multivariate analyses. Yet, a 1% decrease in haemoglobin A1c, was associated with a 9% risk reduction for MI (CI 5%-14%, p=0.001), and a 32% risk reduction for LEA (CI 21%-40%, p =<0.0001) in multivariate models. **Conclusions**: Hyperglycaemia at diagnosis of diabetes is associated with the risk of macrovascular complications to a greater extent than %S or %β. This suggests that once diabetes develops, insulin resistance *per se* no longer increases risk, and that glucose-induced damage predominates.

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FASTING INSULINAEMIA AND MYOCARDIAL INFARCTION RISK IN A GENERAL BELGIAN POPULATION

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Searching for new cardiovascular (CV) risk factors (RF) is warranted, since patients with myocardial infarction (MI) may not exhibit conventional RF (high cholesterol, smoking, hypertension, diabetes and CV family history). Patients with MI often present with dyslipidaemia, hyperglycaemia, android obesity and/or secondary hyperinsulinaemia, a marker for insulin resistance. Previous population studies yielded controversial results as to whether fasting insulinaemia (Ins) is an independent RF for MI. **Aims**: To assess the relationship between Ins and MI in a general Belgian population. **Subjects and Methods**: CV event occurrence (MI or cardiac death) was recorded between 1984 and 1996 in 1948 Belgian subjects from Luxembourg province, who entered the MONICA study. Subjects with previous MI (n=12) or diabetes (n=58) were excluded. This population random sample recruited men and women aged 35-64 years in 1984. **Results**: At baseline, conventional CV RF were highly prevalent (%): cholesterol >250 mg/dl (46); smoking (34); hypertension (16) and BMI>30 (16). Fasting values were: LDL-C 170, HDL-C 55, triglycerides 127, and glycaemia 90 mg.dL^{-1}. Fasting Ins (median: 11 μU.mL^{-1}) was negatively correlated with HDL and positively with triglycerides, glycaemia, blood pressure and BMI. The latter 5 CV RF prevalence was proportional to the quintile of Ins. A first CV event occurred over 12 years in 76 subjects, with a cumulative incidence of 7.6 and 1.4% in men and women respectively. CV risk was related to the number of clinical RF (i.e. male gender, age>55, smoking and hypertension). Fasting Ins, both in uni- and multivariate analysis, was not found to be a significant CV RF, neither in men nor in women. Low HDL-C (<55) was observed in 77% (men) and 35% (women), and HDL-C was the most powerful predictor of first MI from the lipid profile. Low (*vs.* high) HDL-C was associated with increased CV risk in both men (7.5 *vs.* 3.0) and women (3.0 *vs.* 0.3%). **Conclusions**: Fasting insulinaemia did not turn out as independent CV RF in this general population. Individual risk for MI was closely related to number of clinical RF, whilst HDL-C proved the strongest biological predictor of a first CV event

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INSULIN RESISTANCE AND GLUCOSE TOLERANCE ARE NOT RELATED TO LEFT VENTRICULAR MASS IN HUMANS

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Aims: Insulin has been claimed to promote cell growth and, more specifically, to favour left ventricular hypertrophy in humans. We investigated the relationship between left ventricular mass (LVM) and insulin sensitivity/hyperinsulinaemia in non-diabetic subjects with diverse grades of LVM. Materials and methods: We studied three groups of subjects: a) 21 patients with essential hypertension, b) 16 obese normotensive individuals and c) 13 healthy (normotensive, lean) subjects. LVM was quantitated by M-mode echocardiography. Oral glucose tolerance (by OGTT) and insulin sensitivity (by the euglycaemic insulin clamp, 7 pmol·min^{-1}·m^{-1}) were performed in all. Results: By univariate regression analysis on the whole data set, insulin sensitivity was inversely (r=0.35, p=0.02), and the insulin area-under-curve (AUCi) directly (0.60, $p<0.0001$), related to the body mass index. A larger LVM was significantly associated with older age (r=0.41 $p<0.003$) and higher systolic blood (r= 0.49, $p<0.0004$) and diastolic (r=0.30, $p<0.04$) blood pressure levels, but not with insulin sensitivity or AUCi. However, LVM was directly related to the glucose area-under-curve (AUCg) during the OGTT (r=0.31, $p<0.003$). In a multiple regression model including all the significant physiologic co-variates of LVM (*ie*, age, BMI, gender, and blood pressure), neither AUCi nor AUCg nor insulin sensitivity was significantly related to LVM, whose only independent determinants remained systolic blood pressure and BMI. These variables together explained 38% of the total LVM variance. Conclusion: Insulin sensitivity, hyperinsulinaemia, and glucose tolerance are not independent determinants of LVM in non-diabetic humans.

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AN ACUTE ADMINISTRATION OF L-ARGININE IMPROVES ENDOTHELIAL FUNCTION AND INSULIN SENSITIVITY IN PATIENTS WITH MICROVASCULAR ANGINA

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Aims: To evaluate whether an increment in nitric oxide (NO) availability by a continuous endovenous infusion of its precursor, i.e. L-arginine can improve NO induced-vasodilation and peripheral insulin sensitivity in patients with angina and angiographically normal coronary arteries (MA). **Materials and Methods**: Nine patients with MA underwent a continuous infusion of L-arginine (0,125 gr/min) or saline for 120 min, in randomised order. Sixty minutes after L-arginine or saline infusions, an intravenous insulin bolus (0.1 U/kg) combined to a euglycemic clamp was performed. **Results**: During the first hour of the test, mean blood pressure decreased by 8% ($p<0.01$), forearm blood flow increased by 25% ($p<0.05$) and cyclic GMP levels, second messanger of nitric oxide, increased by 24% ($p<0.05$) with L-arginine infusion while all parameters remained similar to baseline with saline infusion. During the same period, insulin, C-peptide, and glucose levels remained unchanged in both tests. After insulin bolus, Δ area under the curve (ΔAUC) of nitrate and nitrite levels, end products of NO metabolism ($NO2^-/NO3^-$), increased five fold (60.82±19.17 vs -14.77±31.8 μmol/l, $p<0.05$) and glucose infusion rate (GIR), an index of insulin sensitivity, enhanced by 32% (282±27 vs 213±23 mg/kg.0-60 min, $p<0.05$) during L-arginine infusion compared to saline. There was a positive and significant correlation between the AUC of GIR and the ΔAUC of $NO2^-/NO3^-$ ($r=0.67$, $p<0.0001$). **Conclusions**: An increment in NO availability improved non-insulin and insulin induced-endothelial function and insulin sensitivity. These data suggest that an impairment in NO activity could contribute to determine insulin resistance in patients with MA.

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Troglitazone, hypertension and postprandial vasodilation in type 2 diabetes.

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Introduction: Insulin (INS) mediated vasodilation is disturbed in type 2 diabetes, probably caused by an impaired direct vasodilator effect of INS or a defect in INS mediated glucose uptake resulting in decreased peripheral vasodilation. Both defects might contribute to hypertension in type 2 diabetes. The individual contribution of either pathway is unclear. This randomised, double blind placebo (Pl) controlled trial was conducted to study the effect of troglitazone (Tr), an INS enhancer, on blood pressure (BP) and postprandial vasodilation in type 2 diabetes with hypertension.
Methods: All patients followed a weight maintaining and sodium/potassium constant diet. Antihypertensive treatment and metformin was stopped 4 weeks prior the study, oral antidiabetics were continued. At base-line and after 6 weeks treatment with Tr. (400mg od) or Pl. glucose and insulin (fasting and during a 3hr meal tolerance test, MTT), 24-hr BP (Spacelab), Cardiac output (CO) and forearm blood flow (FBF) (venous occlusion plethysmography),basal and during MTT, were monitored. Total peripheral resistance (TPR, in arbitrary units, AU) was calculated as MAP/CO.
Characteristics: Tr (n=14) vs Pl (n=7) (mean±SD) age 61±6 vs 60±7yr, male/female 7/7 vs 4/3, BMI 28.7±5.0 vs 26.9±4.8kg/m^2, HbA_{1c} 7.7±1.0 vs 8.0±1.2% (all $p>0.05$).
Results: After Tr-treatment FBG and INS decreased: 11.8±3.2 vs 9.8±3.1mmol/l and 19±8 vs 11±4mU/l, resp (all $p<0.05$). These values were unaltered after Pl: 11.8±4.4 vs 10.6±3.3 mmol/l and 10±2 vs 10±4mU/l ($p>0.40$), resp. After Tr. the 24-hr diastolic BP, TPR and basal FBF decreased: 88±7 vs 82±6mmHg, 21±4 vs 19±3AU and 3.4±1.7 vs 2.5±1.4ml/dl/min (all $P<0.05$). These values did not change after Pl: 89±11 vs 87±15 mmHg, 22.0±5.8 vs 20±7AU and 2.4±0.9 vs 2.2±1.1 ml/dl/min, resp ($p=0.4$). INS levels and FBF after a standard meal (expressed as 180min AUC) were lower after Tr.: 10100±6542 vs 6749±3673mU/l ($p<0.01$) and 1412±627 vs 961±516 ml/dl/min ($p<0.05$) and did not change after Pl: 6143±1438 vs 7061±1786mU/l ($p=0.06$) and 1120±490 vs 1093±555 ml/dl/min ($p=0.83$). In both groups postprandial hyperglycemia was unaltered.
Conclusion: Improvement of insulin sensitivity by Tr. was associated with a reduction in diastolic BP, probably caused by a decrease in TPR. As basal FBF decreased, vasodilation most likely occurred in organs other than skeletal muscle. The parallel decrease in postprandial vasodilation and hyperinsulinemia, while postprandial hyperglycemia did not change, suggests that this vasodilation is caused by a direct vascular action of insulin and not by its glucose lowering effect.

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INSULIN RESISTANCE SYNDROME DOES NOT DISTURB NORMAL NOCTURNAL BLOOD PRESSURE FALL IN TYPE 2 DIABETIC PATIENTS.

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The attenuated reduction of night blood pressure seems to be associated with higher cardiovascular morbidity. Aim of the study was to examine the relation between insulin resistance and diurnal blood pressure rhythm in obese subjects with borderline hypertension recently diagnosed. Material and methods: investigated group consisted of 39 obese patients aged 18-44 years (mean 30,9±1,5 SE) and in 10 age-sex-matched non-obese healthy subjects. Patients did not take any medication and investigation was conducted in the period when blood pressure was normal (JNC-VI criteria). Measurements of body mass index (BMI), waist/hip ratio (WHR), cholesterol total, LDL, HDL, triglycerides, haemoglobin HbA1c were made and oral glucose tolerance test (OGGT) was performed in all subjects. Insulin sensitivity was estimated by the insulin-suppression test, i.e., by measuring the steady-state plasma glucose (SPG) levels achieved et the end of infusion of somatostatin, insulin and glucose. Test accuracy was estimated by C-peptide and insulin concentrations. Diurnal blood pressure rhythm was estimated by 24-hour blood pressure monitoring with SpaceLabs device. Results: oral glucose tolerance test revealed 11 cases of type 2 diabetes mellitus (DM) previously not diagnosed. In this group mean SPG level was 214,2±7,4 mg/dl. Non-diabetic obese subjects were divided according to SPG levels into subgroups: insulin resistant (IR) group - 14 patients with mean SPG level 174,5±5,1 mg/dl and insulin sensitive (IS) group -14 patients with mean SPG level 104,9±8,9 mg/dl. SPG level in healthy subjects (H) was 62,8±3,3 mg/dl. In diabetic group patients were older, WHR and total cholesterol, LDL cholesterol, triglycerides and HbA1c levels were higher than in IS and H group but similar to values in IR group. Blood pressure values registered in 24-hour record were within the norm in all the groups however in DM patients day and night diastolic and MAP pressure was significantly higher than in the control group. In diabetic patients correlation between SPG levels and diastolic ($r=0,69$, $p=0,02$) and MAP ($r=0,66$, $p=0,03$) pressure during the night was observed. Normal and similar (>10%) nocturnal systolic and diastolic blood pressure fall was observed in all investigated groups. Conclusion: insulin resistance syndrome in type 2 diabetic patients with borderline hypertension does not disturb the nocturnal fall of blood pressure.

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SERUM INSULIN AND C-PEPTIDE IN DEFINED, URBAN POPULATION: SELECTIVE CORRELATIONS WITH HEMOSTATIC FACTORS

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Increased serum concentration of insulin (RIA) and C-peptide (RIA) may be used as the marker of insulin resistance - a risk factor for ischemic heart disease. Its influence has multiple mechanism. One of less studied is the correlation between insulin and C-peptide and hemostatic factors. Therefore in a well difined, representative urban population the study was undertaken aimed at cross-sectional determination of insulin and C-peptide together with the serum level of fibrynogen (F), tissue plasminogen activator (t-PA), inhibitor of tissue plasminogen activator (PAI-1), von Willebrand factor (vW) and factor VII (f.VII). In the population under study composed of 269 selected with the demographic layers proportional metod the average fasting insulin level was 9.33 mu/l (SD 6.24), C-peptide 1.94 ng/ml (SD 0.87), F - 241.4 mg/dl (SD 50.5), t-PA 8.71 ng/ml (SD 6.31), PAI-1 12.25 ng/ml(SD 9.44), vW 121.1% (SD 85). In 41 cases the fasting insulin level was above normal (15mu/l) level, this was accompanied by increase C-peptide level. In this subgroup the average levels of fibrinogen, t-PA, PAI-1 and of vW factor were higher than in general population under study. In the whole group statistically valid correlations between C-peptide levels and serum concentration of fibrinogen ($p=0.003$), the antigen and activity of PAI-1 ($p.=0.0001$) and of t-PA ($p.=0.0001$) were found. Important correlation existed also between serum levels of insulin and of fibrinogen, t-PA and vW factor. Conclusion: distribution of levels and correlations of insulin and C-peptide on one hand and of the hemostatic factors under study on the other side as found in the difined population may represent the important mechanism in the pathogenesis of ischemic heart disease. Its early diagnosis may serve as the basis of intervention.

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VITAMIN C AND INSULIN RESISTANCE IN ESSENTIAL HYPERTENSION

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Aims: We tested whether intra-arterial, high-dose (12 mg/min) vitamin-C (Vit-C) infusion, by improving nitric oxide (NO)-dependent vasodilatation, attenuates the insulin resistance of skeletal muscle tissue in 9 patients with essential hypertension (EH). Materials and Methods: Study I) The effect of Vit-C on acetylcholine (Ach, 4.5 $\mu g \cdot min^{-1} \cdot dl^{-1}$) induced vasodilatation was measured (by plethysmography). Study II) Forearm blood flow (FBF) and glucose and oxygen exchange (A-V differences) were measured in both forearms (infused and control) at baseline, 80 min into a euglycaemic hyperinsulinaemic clamp, and following Vit-C infusion (only into the infused forearm) for another 20 min. Results: Study I) Intrabrachial Ach infusion produced a stable increase in FBF from 2.6±0.3 to 10.6±2.1 $ml \cdot min^{-1} \cdot dl^{-1}$; when Vit-C was co-infused, FBF showed a further rise to 13.4 $ml \cdot min^{-1} \cdot dl^{-1}$ ($p<0.03$ *vs* Ach), indicating an improvement in local NO availability. Study II) Basal FBF (infused: 2.9±0.2, control: 2.6±0.3 $ml \cdot min^{-1} \cdot dl^{-1}$) was not changed by the systemic insulin administration, whereas it increased by 26% only in the infused forearm when Vit-C was added (infused: 3.7±0.7, control: 2.8±0.6 $ml \cdot min^{-1} \cdot dl^{-1}$, $p<0.02$). Insulin administration stimulated whole-body glucose disposal to 20±2 $\mu mol \cdot min^{-1} \cdot dl^{-1}$, confirming the presence of marked insulin resistance. Basal forearm glucose uptake (infused: 0.24±0.10 and control: 0.22±0.10 $\mu mol \cdot min^{-1} \cdot dl^{-1}$) was similarly stimulated by systemic insulin infusion (infused: 2.11±0.42 and control: 2.06±0.43 $\mu mol \cdot min^{-1} \cdot dl^{-1}$). When intrabrachial Vit-C was added, no difference in glucose uptake was observed between the two arms (infused: 2.37±0.44 and control: 2.36±0.53 $\mu mol \cdot min^{-1} \cdot dl^{-1}$). Oxygen uptake was similar in the two forearms (infused: 9.7±0.7 control: 9.6±1.1 $\mu mol \cdot min^{-1} \cdot dl^{-1}$), and was not changed by either insulin or Vit-C.

Conclusion: In the deep forearm tissues of patients with EH and insulin resistance, an acute increase in endothelial-derived NO availability, obtained with pharmacological doses of Vit-C, potentiates insulin-mediated vasodilatation but does not improve insulin-mediated glucose uptake. Thus, the vascular insulin resistance of EH is unlikely to be responsible for the metabolic insulin resistance.

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EFFECTS OF ANGIOTENSIN II AND AT_1 & AT_2 RECEPTOR BLOCKADE ON GLUCOSE AND LIPID METABOLISM IN VIVO AND IN VITRO

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Clinical studies have shown that systemic infusion of angiotensin II (AII) increases insulin sensitivity but the underlying mechanism (eg redistribution of blood flow and/or a direct biochemical effect via AT_1 or AT_2 receptors) is unclear. Aims: To evaluate the metabolic effects of AII ± AT_1 (L-158,809) and AT_2 (PD-123,319) receptor blockade in vivo and in vitro. Methods: (I) Groups of fructose-fed insulin-resistant SD rats and chow-fed controls were gavaged with L-158,809 300 μg/kg/d or vehicle for 7 days prior to measuring the effects of AII infusion (20 ng/kg/min i.v) on whole-body insulin sensitivity (insulin suppression test [IST]) and total triglyceride (TG) secretion rate (TG-SR); (II) The effects of AII (10^{-7}-10^{-9}M) ± AT_1/AT_2 blockade on insulin-stimulated ^{3}H-glucose uptake in isolated L6 myotubes was measured in vitro. Results: Short-term treatment with L-158,809 (n=16) vs vehicle (n=16) had no significant effect on fasting serum glucose and TG levels and oral glucose tolerance in fructose-fed rats: eg AUC_{GLU} post-OGTT (day 5) was 20 ± 0.4 vs 19.2 ± 0.5 mmol.h/L. AII infusion had no significant effect on whole-body insulin sensitivity either in L-158,809-treated or vehicle-treated fructose-fed rats: eg, SSPG values during the IST after co-infusion of AII or saline were 9.6 ± 0.3 (n=7) vs 7.1 ± 0.6 (n=7) mmol/L (NS), respectively, in rats pretreated with L-158,809. Infusion of AII had no significant effect on TG-SR: eg, in L-158,809-treated rats TG-SR was 24.01 ± 2 (AII) vs 20.7 ± 1.4 (saline) mg/100g/hr. Dose-response curves for insulin-mediated glucose uptake in L6 cells were unaffected by co-incubation with AII in doses of 10^{-7} – 10^{-9}M; neither E_{max} nor the insulin $C_e(50)$ was influenced by AII or the AT_1/AT_2 antagonists. Conclusions: AII had no direct effect on insulin-stimulated glucose uptake in L6 cells, nor any effect on glucose and lipid metabolism in a rodent model of dietary-induced insulin resistance, suggesting that the insulin sensitizing effect of AII in humans is predominantly an indirect effect, presumably due to increases in limb blood flow.

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ALDOSTERONE DECREASES BOTH INSULIN RESPONSIVENESS FOR GLUCOSE TRANSPORT AND INSULIN BINDING IN U-937 CELLS.

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We have recently reported that aldosterone (ALD), the major mineralocorticoid in humans, inhibited insulin receptor (IR) mRNA levels in U-937 cells by a mechanism involving the mineralocorticoid receptor (MR). The **Aim** of the present work was investigate whether ALD-inhibition of IR gene expression could implicate (i) alterations in both insulin binding and insulin-stimulated glucose transport in these cells and also (ii) a homologous regulation of MRs by this hormone. **Methods:** 10^{-9} M ALD was directly applied to the cells for 48 h. **Results:** Insulin binding assays indicated that ALD treatment caused a clear reduction (34%) in IR capacity (15611 ± 1791 vs. 23616 ± 7227 sites per cell) although the effect was only statistically significant at insulin concentrations below 1.25×10^{-9} M. In addition, IR affinity resulted unaltered (Kd (10^{-9} M): 1.20 ± 0.09 vs. 1.30 ± 0.17). Measurements of glucose transport in these cells, in the absence and presence of increasing concentrations of insulin (10^{-11}-10^{-7} M), indicated that ALD did not decrease basal levels of glucose uptake (642 ± 69 vs. 603 ± 50 fmol/min per 10^6 cells). However, ALD-treatment decreased (25%) the maximal insulin-stimulated glucose transport at 10^{-8} M insulin. Moreover, the ED_{50} value was greater in ALD treated (0.40×10^{-9} M) than in untreated cells (0.15×10^{-9} M). Thus, ALD caused a decrease in insulin responsiveness in terms of glucose transport. In addition, whole cell ALD binding assays indicated that treatment with ALD clearly reduced (48%) MR capacity (1186 ± 207 vs. 2275 ± 247 sites per cell; $p<0.05$), without altering receptor affinity (Kd (10^{-9} M): 2.30 ± 0.40 vs. 1.60 ± 0.17). Northern blot assays, using as probe the 1.3-Kb human MR-specific EcoRI fragment of the pRShMR clone, showed the presence in these cells of a unique specie of MR mRNA of around 4.5-Kb in size. The levels of this RNA decreased (50%) after ALD treatment. This inhibitory effect of ALD on MR mRNA levels was reversed by the ALD antagonist, spironolactone. **In conclusion**, these results indicate that the inhibition by ALD of IR gene expression in U-937 cells implicates decreases in both insulin responsiveness and insulin binding. These effects are associated with a homologous down-regulation of MR expression in these cells.

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ALTERED CATECHOLAMINE METABOLISM IN OFFSPRING OF PROTEIN RESTRICTED RATS

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Aim: To test the hypothesis that catecholamine metabolism is altered with early growth retardation in the rat, since preliminary data from humans and rats suggests that it may be. **Materials and Methods:** Wistar rats were fed either a 20 % protein ('control') diet or an isoenergetic 8 % protein diet ('MLP') during pregnancy and lactation. Male offspring were weaned on a standard laboratory chow and at 12 weeks of age blood was sampled from them in the fed and 24-hour-fasted states for catecholamine measurements. Some of the rats were then killed and their epididymal adipocytes isolated and blotted using antibodies against rat ß1-adrenoreceptors. **Results:** In the fed state mean (S.E.M.) plasma adrenaline concentrations for control (n=12) and MLP (n=11) rats were: 0.78 (0.13) v. 1.42 (0.16) nM (P<0.01; Mann Whitney U test), respectively. Equivalent noradrenaline concentrations were: 2.78 (0.27) v. 3.61 (0.22) nM (P<0.05). After 24 hours starvation plasma adrenalines of controls rose to become similar to those of MLP rats: 1.10 (0.13) v. 1.42 (0.23) nM (P=0.27), respectively. Noradrenaline concentrations rose in both groups to become similar: 4.82 (1.02) v. 5.15 (0.75) nM (P=0.30). Adipocyte ß1-adrenoreceptors were raised 2.6 (0.4) fold in MLP rats (n=4 both groups; P<0.05). **Conclusion:** These results are consistent with there being an alteration in catecholamine metabolism in rats growth restricted in early life by exposure to maternal dietary protein restriction.

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PLASMA CORTISOL BINDING GLOBULIN (CBG) LEVELS ARE ASSOCIATED WITH INSULIN SECRETION

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Aim: It has been shown *in vitro* that insulin is a potent inhibitor of both cortisol binding globulin (CBG) and sex-hormone binding globulin (SHBG) secretion. To further investigate this effect of insulin *in vivo* we studied three groups of lean (*L*), obese (*Ob*) and obese with glucose intolerance (*Obl.*) otherwise healthy subjects. **Material and Methods:** Plasma total CBG, glycosylated CBG, SHBG and insulin sensitivity (S_I) and secretion (AIRg) (minimal model method) were measured in 16 *L*, 10 O and 11 *Obl.* subjects. **Results:** Plasma total CBG concentration (p=0.016) and glycosylated CBG levels (37.3 ± 5.2 vs 31 ± 3.9 mg/l; p=0.018) were significantly increased in *Obl* subjects. Plasma CBG correlated with fasting glucose levels (r=0.49, p=0.002), HbA_{1c} levels (r=0.35, p=0.03) and with area under the curve of glucose after TTOG (r=0.45, p=0.005), and negatively with AIRg (-0.38, p=0.02). CBG levels did not covariate with S_I. Multiple linear regression analysis showed that only AIRg contributed to the variability of CBG concentration (p=0.03, R^2=0.41). In both men and women, SHBG levels correlated negatively with glucose (r=-0.55, p<0.0001), HbA_{1c} (r=-0.38, p=0.02) and positively with S_I (r=0.65, p=0.003 and r=0.63, p=0.007, in men and women, respectively) but not with AIRg. The disposition index (S_{IAIR}: S_I * AIRg) was significantly decreased in the *Obl* subjects. S_{IAIR} correlated with plasma SHBG levels (r=0.52, p=0.001) and negatively with plasma CBG levels (r=-0.54, p=0.001). **Conclusions:** These data suggest that plasma CBG is a marker of insulin secretion in a similar way as SHBG is a marker of insulin sensitivity. As high plasma CBG levels have been associated with increased incidence of type 2 diabetes, this important issue merits further investigations.

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1,25-DIHYDROXYVITAMIN D_3 INCREASES INSULIN BINDING AND INSULIN-STIMULATED GLUCOSE TRANSPORT IN U-937 CELLS.

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Earlier studies of our laboratory have demonstrated a positive regulation of insulin receptor (IR) mRNA levels by 1,25-Dihydroxyvitamin D_3 (VD) in U-937 cells, a human promonocytic cell line with many of the characteristics of circulating blood monocytes. The **Aim** of the present work was examine whether this stimulation of IR gene expression by VD could involve regulation of both insulin binding and insulin sensitivity in terms of glucose transport. In addition, we also determine whether these VD actions could be associated with increased VD receptor mRNA levels. **Methods:** 10^{-8} M VD was applied to the cells for 24 h. **Results:** Insulin binding assays indicated that treatment with VD clearly increased the number of both high affinity (0.33 ± 0.01 vs. 0.17 ± 0.01 ng/ml; p<0.05) and low affinity insulin sites (2.42 ± 0.33 vs. 1.28 ± 0.11 ng/ml; p<0.05) while the affinity of both types of sites: K_{D1}(10^{-9} M) (0.95 ± 0.05 vs. 0.90 ± 0.05) and K_{D2}(10^{-9} M) (15.0 ± 2.0 vs. 11.1 ± 1.0) remained unaltered. Thus, the increased binding was caused by a 1.8-fold increase of the total number of IRs per cell. Glucose transport assays indicated that insulin (10^{-11}-10^{-7} M) stimulated the uptake of glucose in a dose-dependent manner in both untreated and VD-treated cells. Treatment with VD had no significant effect on basal glucose uptake (669 ± 26 vs. 603 ± 50 fmol/min per 10^6 cells). However, the maximal uptake, at 10^{-8} M insulin, was 1.2-fold higher in VD-treated than in untreated cells, without changes in the ED_{50} (10^{-9} M) value (0.08 ± 0.009 vs.0.15 ± 0.017). Thus, the addition of VD caused a maximal increase of 1.2-fold in insulin sensitivity to glucose transport in these cells. Northern assays using a specific VD receptor [^{32}P]-labelled probe (the 2.1-Kb human VDR EcoRI fragment of pGEM-3 clone) revealed the presence of a unique specie of VD receptor mRNA of approximately 4.6-Kb in size. Treatment with VD caused around 1.5-fold increase in the levels of this RNA. **In conclusion**, the present results demonstrate that the increase in IR mRNA levels induced by VD leads to an increase in IR number per cell and concomitantly to an increase in cellular sensitivity to insulin. These effects seem to be associated with enhanced VD receptor mRNA levels.

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IS CORTISOL AXIS DYSREGULATION INVOLVED IN THE PATHOGENESIS OF TYPE 2 DIABETES ?

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Aims and methods: To elucidate dysregulation of the cortisol (HPA) axis as a potential mechanism of early insulin resistance and type 2 diabetes, 15 individuals with (relatives=R) and 15 without (controls=C) family history (first-degree relatives) of type 2 diabetes were examined. R and C were matched for age (R 32.5±1.9 vs C 32.7±1.9 yr, mean±SE), BMI (24.2±0.9 vs 23.6±0.7) and sex (M/F 8/7). All subjects underwent a 75 g OGTT and a hyperinsulinemic (≈ 110 mU/L) euglycemic clamp. A 24 h profile of salivary cortisol was assessed. Serum cortisol levels were measured during dexamethasone suppression test (3.5 µg/kg orally) as well as during ACTH (Synacthen®, 1µg i.v) and CRH (1µg/kg i.v) stimulation. **Results:** Fasting serum glucose, insulin and HbA1c did not differ between R and C and neither did insulin sensitivity index (M-value/plasma insulin) assessed during clamp (9.2±1.3 vs 9.7±0.9). 24 h urinary cortisol was similar in both groups and so was morning serum cortisol (R 464±34 vs C 531±30 nmol/L) also following the oral dexamethasone suppression test (318±41 vs 330±39 nmol/L). Stimulation with ACTH as well as with CRH produced lower serum cortisol in R compared with C (by ≈15%, p<0.05). However, plasma ACTH levels after CRH stimulation did not differ between the groups, suggesting an impaired adrenal cortex sensitivity to ACTH. The rise in salivary cortisol from evening to morning was smaller in R (by≈40%, p<0,05), supporting a blunted variability in HPA-axis function. Post-dexamethasone serum cortisol levels were correlated to insulin sensitivity (r=0.43, p<0.03) indicating a link between insulin resistance and glucocorticoid feedback mechanisms. **Conclusion**: Diabetes-prone healthy individuals display alterations in the fine-tuning of the HPA-axis regulation and this may be involved in the development of insulin resistance and type 2 diabetes.

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HYPERACTIVITY OF THE HYPOTHALAMO-PITUITARY-ADRENOCORTICAL AXIS DESPITE NORMAL WEIGHT AND GYNOID FAT DISTRIBUTION IN INSULIN RESISTANT OFFSPRING OF TYPE 2 DIABETES PATIENTS.

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Aims: Both obesity and android fat distribution have repeatedly been associated with hyperactivity of the hypothalamo-pituitary-adrenocortical axis (HPA). It is not clear, whether insulin resistance per se affects HPA activity independent of obesity and android fat distribution.

Methods: We performed a corticotropin releasing hormone stimulation test (CRH 1µg/kg body weight i.v.) and measured both plasma corticotropin (ACTH) and serum cortisol responses during 120 min. 30 female glucose-tolerant offspring of caucasian subjects with type 2 diabetes (FDR) were examined. Glucose tolerance was determined by an oral glucose load (oGTT; 40g glucose/m²) and insulin sensitivity by euglycaemic-hyperinsulinaemic glucose-clamp. 10 insulin resistant women with normal weight and gynoid fat distribution pattern (IRGFD; MCR <6 ml•kg^{-1}•min^{-1}; BMI <25 kg/m²; WHR<0.80) were compared with 10 overweight women with android fat distribution pattern (IRAFD; BMI >25 kg/m², WHR>0,85), matched for insulin sensitivity. 10 insulin sensitive women (MCR >8 ml•kg^{-1}•min^{-1}) with normal weight and gynoid fat distribution pattern served as controls.

Results: Basal plasma ACTH and serum cortisol was not different between groups. However, IRGFD normal weight women had almost the same incremental areas (AUCi) of ACTH and cortisol (pmol/L•min and µmol/L•min) as IRAFD obese women.

	Age	BMI	WHR	MCR	$AUCi_{ACTH}$	$AUCi_{cortisol}$
IRGFD	32±1	23±0.7	0.74±0.01	5.6±0.2	504±103	57±7
IRAFD	36±2	30±0.9	0.86±0.02	5.1±0.3	490±152	50±5
CTRL	33±1	22±0.7	0.76±0.01	9.9±1.4	256± 82	19±2

Conclusion: We describe a subgroup of insulin resistant daughters of parents with type 2 diabetes, where HPA-hyperactivity occurs despite normal weight and gynoid fat distribution. We suggest an independent role of insulin resistance in HPA-hyperactivity.

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Effects of the β_3-Adrenoreceptor Agonist UL-TG 307 on Insulin Sensitivity and Insulin Secretion in Type 2 Diabetic Patients

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In obese rodents administration of β_3-adrenoreceptor β_3-AR) agonists increased thermogenesis and induced weight loss. In animal models of type 2 diabetes treatment with β_3-AR agonistic agents improved whole body-, liver-, and adipose tissue insulin sensitivity and led to an enhanced insulin secretion. We studied the effects of a novel β_3-AR agonist, UL-TG 307, on insulin sensitivity, insulin secretion, lipid metabolism, and body weight in thirteen diet treated male patients with type 2 diabetes. Participants were investigated in a randomized, double-blind placebo controlled cross-over trial consisting of two 14 day administration periods with placebo and UL-TG 307 (24 mg daily). After each treatment period insulin secretion was assessed by an OGTT and insulin sensitivity was measured by an euglycemic hyperinsulinemic glucose clamp. Lipid metabolism was evaluated by measuring non-esterified fatty acid (NEFA) and glycerol serum concentrations at the end of each administration period. Treatment with UL-TG 307 did not improve insulin sensitivity (insulin sensitivity index S_I) nor increased insulin secretion during OGGT (AUC ratio of immunoreactive insulin (IRI) and blood glucose (BG); Tab.). NEFA and glycerol tended to be higher after UL-TG 307, the difference however did not reach statistical significance. Fructoseamine and body weight did not change significantly either. We conclude that two weeks administration of the β_3-adrenoreceptor agonist UL-TG 307 in a daily dose of 24 mg did not lead to any metabolic significant effect in diet treated type 2 diabetic patients.

	Baseline	UL-TG 307	Placebo
S_I (ml/min*m² per µU/ml)	-	2.5±0.6	2.2±0.8
IRI AUC / BG AUC	-	8.8±7.4	8.3±6.4
NEFA (mmol/L)	0.8±0.4	1.3±1.1	1.0±0.6
Glycerol (µmol/L)	197±70	244±115	176±79
Fructoseamine (µmol/L)	324±43	323±55	313±47
Weight (kg)	98±11	99±11	99±11

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DIRECT EFFECT OF TUNGSTATE ON THE BIOSYNTHESIS OF INSULIN IN ISOLATED RAT ISLETS.

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It has been described that administration of tungstate to different animal models of diabetes mellitus leads to a normalization of blood glucose and restores the level of insulin release and content. However, the direct effect of tungstate is unknown. **Aim**: To investigate the action of tungstate in isolated rat islets. **Materials and Methods**: Control rat islets and streptozotocin-damaged islets were cultured in RPMI supplemented medium at different concentrations of glucose (5.5 and 24.4 mM) and tungstate (1, 10 and 100 µM) for 7 days. Studies of cellular viability (ethidium bromide/acridine orange), insulin release and content (RIA), insulin biosynthesis (immunoprecipitation), insulin mRNA (Northern blot), IDX protein levels (Western blot) were performed. Morphometrical analysis and cell replication were also studied. **Results**: The presence of tungstate in the culture medium did not affect cell viability of control islets (>95% survivor). STZ-damaged islets treated later with tungstate showed a less lost of cells than these untreated (80% living cells vs. 30%), suggesting a possible effect on cell replication. Meanwhile insulin release in control islets was not affected, tungstate was able to recover insulin content at high glucose (772 ± 45 µU/islet in control vs. 1306 ± 118 in 1µM tungstate). These results are also done in STZ-damaged islets. Protein levels of insulin highly increase at low glucose (2-fold) and more slightly at high glucose. Nevertheless insulin mRNA was diminished and protein levels of insulin specific transcrition factor IDX-1 was poorly affected by tungstate. **Conclusions:** The effect of tungstate in vitro over control islets relays on insulin biosynthesis rather than in β-cell replication; nevertheless in STZ-damaged islets it may be act through β-cell replication. These results are consistent with those obtained in vivo, where the effect of tungstate over β-cell number increase is observed in diabetic but not in healthy animals. The mechanisms implicated in insulin biosynthesis are not well elucidated and other insulin transcription factors as well as post-transcriptional factors must be investigated.

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ENDOCRINE AND EXOCRINE PANCREATIC FUNCTION IN DIABETIC SUBJECTS IN BANGLADESH

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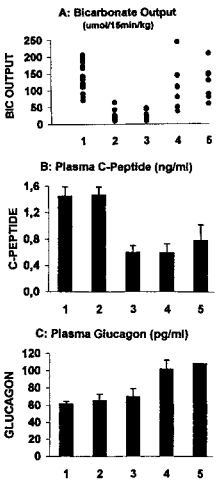
Specific types of diabetes mellitus and chronic pancreatitis (CP) were described in tropical regions: non-alcoholic CP: Tropical Chronic Pancreatitis (TCP); CP with diabetes as key clinical sign: Fibro-Calculus Pancreatic Diabetes (FCPD); lean type diabetes without CP: Protein-Deficient Diabetes Mellitus (PDDM). Aim: to prospectively compare (a) exocrine and (b) endocrine pancreatic function in untreated patients with matched healthy controls. Methods (mean±SEM): ① 27 controls (HbA1c=5.3±0.1), ② 8 TCP (5.7±0.1), ③ 14 FCPD (13.6±1.2), ④ 13 PDDM (15.6±0.8) and ⑤ 11 type II diabetics (NIDDM) (11.5±0.9) underwent (a) a secretin test (duodenal intubation, interval aspiration of secretions at baseline and during 60 min. i.v. infusion of secretin 250 ng/kg) with measurement of bicarbonate output, and (b) an arginine test (baseline and stimulated blood samples in 15 min. intervals, using an i.v. infusion of 1.4 mval/kg Arg-HCl during 30 min.) with assessment of plasma C-peptide and glucagon. Results: (Fig. A) Bicarbonate output of 70 µmol/15min/kg could discriminate every single patient with CP (group ②③) from controls. (Fig. B) Arg-HCl stimulated plasma C-peptide levels expressed as incremental response were significantly decreased in all groups with diabetes (③④⑤) compared to controls and patients with CP only (②) ($p<0.05$). (Fig. C) Stimulated glucagon levels were significantly different in FCPD (③) than in other groups with diabetes (④⑤) ($p<0.05$) and were in the same range of controls and TCP. Conclusion: In addition to generalized pancreatic damage, selective B-cell impairment may be involved in the development of diabetes in tropical pancreatitis.



PS 51
Leptin I

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LEPTIN DOES NOT DETERMINE INSULIN ACTION EXCEPT IN INSULIN RESISTANCE

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Aims: Leptin is supposed to be involved in the pathogenesis of obesity-associated insulin resistance.

Materials and Methods: We therefore directly quantified insulin sensitivity by hyperinsulinemic-euglycemic glucose-clamp (Metabolic clearance rate [MCR]) in 156 healthy, glucosetolerant offspring of patients with type 2 diabetes. Additionally, serum leptin and percentage of body fat (impedance technique) were measured.

Results: 55 offspring were defined as insulin resistant (IR) with a MCR<6ml/kg/min, 58 with a MCR>8.2 ml/kg/min as insulin sensitive (IS). Serum leptin levels correlated with percentage of body fat (r=0.75) and insulin sensitivity (r=0.33). IR had higher leptin levels than IS (21 vs. 8 ng/ml). Even when expressed as leptin per percentage of body fat, IR remained higher (0.68 vs. 0.39 ng/ml/%fat). MCR and leptin levels were correlated in IR (r=0.36) but not in IS (0.1). IR and IS were further subdivided in lean and obese with a BMI 25kg/m^2 as cut-off point. 16 IR and 43 IS were lean, whereas 39 IR and 16 IS were obese. Leptin levels were significantly different between lean and obese IR (11 vs. 25 ng/ml) and for the corresponding IS (6 vs. 15 ng/ml). During hyperinsulinemic-euglycemic glucose-clamp, leptin levels remained constant in all four subgroups.

Conclusions: Leptin levels are not influenced by acute hyperinsulinemia. Leptin levels mainly depend on percentage of body fat and do not explain different levels of insulin sensitivity. Neither do lean IR have high leptin levels nor do obese IS have low leptin levels that could explain their insulin resistance or sensitivity. Only before a background of insulin resistance are higher leptin levels associated with lower MCR. In IR higher leptin levels are due to higher percentage of body fat but in addition due to higher body fat-derived leptin secretion.

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THE INSULIN RESISTANCE INDEX AND LEPTIN ARE INFLUENCED BY FATTY ACIDS CHAIN LENGTH AND DEGREE OF SATURATION

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Aim: Plasma free fatty acids (FFA) levels are known modulators of both insulin secretion and action. Recently, the insulinotropic potency of FFA have been related to their chain length and degree of saturation in animals. We therefore examined the relationship among fasting individual FFA, insulin and leptin concentrations in humans. **Material and Methods:** In ninety-nine healthy women, body composition, fasting plasma individual FFA (from C:14 to C:24), insulin and leptin concentrations were measured. **Results:** The mean ± SD body mass index (BMI) was 23.5 ± 3.9 kg/m^2; age 38.2 ± 9.4 years. Only palmitoleic acid and linoleic acid correlated with fasting insulin (r=0.26, p=0.01, and r=0.21, p=0.34) and with fasting insulin resistance index (FIRI) (r=0.26, p= 0.008 and r=0.22, p=0.026). Palmitoleic acid also correlated with fat mass and leptin concentration (r=0.25, p=0.011 and r=0.20, p=0.048). In a multiple linear regression analysis, BMI (p=0.00001) and the percentage of total saturated FFA (p=0.017) independently contributed to the variability of FIRI (R^2=0.26). Leptin levels were also independently predicted by BMI (p<0.00001) and the percentage of saturated FFA (p=0.025)(R^2=0.53). Finally, in another model, both BMI (p<0.00001) and palmitoleic acid (p=0.038) independently contributed to the variance of FIRI (R^2=0.27).
Conclusions: The fasting insulin resistance index and leptin levels are influenced by fatty acids chain length and degree of saturation. It is tempting to speculate that an excessively high ratio of saturated to unsaturated fatty acids in the plasma pool might promote hyperinsulinism.

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SERUM LEPTIN AND THE MUSCULAR COMPARTMENT

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Aim: Large variation in circulating leptin concentrations at similar levels of adiposity indicates that factors other than fat mass may be important in regulating serum leptin. Recent experimental works in mice demonstrated that leptin is also synthesized by muscle cells. **Material and methods:** We evaluated body composition (through bioelectric impedance and anthropometrical parameters), insulin resistance, and leptin levels in 140 men and 99 women. **Results:** Serum insulin, the fasting insulin resistance index (FIRI) and serum leptin levels were significantly increased in men in the highest quintile of fat-free mass (FFM). Leptin levels *positively* correlated with FFM in men (r=0.24, p=0,004) but not in women (r=0.02, p=NS). Leptin also correlated with mid-arm muscle circumference (MAMC) and area (MAMA) (r=0.24 and r=0.23, respectively, p=0.004 and p=0.009) in men but not in women. As expected, serum leptin was strongly associated with fat mass (FM) (r=0.58 and r=0.71, p<0.0001 in men and women, respectively) and FIRI (r=0.48 and r=0.53, p<0.0001 in men and women, respectively). In a multiple linear regression analysis in a stewise manner to predict leptin levels, FM, FFM, and FIRI independently contributed to 32%, 6% and 3% of the variance in serum leptin levels in men (p<0.00001, p=0.022 and p=0.029, respectively). In women, FM (49%), FIRI (3.6%) and waist-to-hip ratio (2.4%), but not FFM, explained 55% of the variance in serum leptin (p<0.00001, p=0.0045 and p=0.04, respectively). **Conclusions:** the muscle compartment contributes to the variability of serum leptin levels in men. Whether insulin resistance at this level mediates an increased production of leptin merits further research.

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PLASMA LEPTIN CONCENTRATIONS IN INSULIN RESISTANT AS COMPARED TO INSULIN SENSITIVE HEALTHY VOLUNTEERS

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Leptin , the product of the OB gene is produced in adipose tissue and studies in human beings emphasize the presence of elevated concentrations in obese individuals. Plasma insulin concentrations are often increased in obese individuals , raising the possibility that the relationship between obesity and leptin is mediated via the hyperinsulinemia associated with obesity. To test this hypothesis 421 healthy subjects , factory workers , have been examined and divided into 4 quartiles on the basis of their insulin area after OGTT, as index of insulin resistance. The comparison (mean± S.E.) has been done between the subjects in the 4th quartile , with the highest insulin response to the glucose challenge and the subjects in the 1st quartile with the lowest insulin response . Leptin has been measured by radioimmunoassay Results: hyperinsulinemic and insulin resistant subjects (quartile 4: n=101; age 53±0.9 y.; BMI 28.4±0.4 Kg/m^2), compared to the insulin sensitive subjects (quartile 1: n=100; age 50±0.7 y.; BMI 24.5±0.3 Kg/m^2) show significantly higher levels of plasma insulin, plasma glucose, triglyceride, lower levels of HDL cholesterol, higher blood pressure. Plasma leptin concentration is significantly higher in the hyperinsulinemic and insulin resistant group : 12.60±0.85 vs 8.53±0.56 ng/ml; p<0.001). Leptin is significantly correlated with BMI, gender and plasma insulin; no relationship was found between leptin and age or W/H ratio. The multiple regression analysis shows a relationship between plasma leptin and insulin independent of BMI and gender. Conclusion: these data indicate the presence of a relationship between leptin and hyperinsulinemia independent of obesity.

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SEX DIMORPHISM OF PLASMA LEPTIN LEVELS AND INSULIN RESISTANCE IN HYPERTENSIVE SUBJECTS

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Aims we investigated gender differences in fasting plasma leptin (FPL) levels and insulin resistance between hypertensive and normotensive subjects.

Material and methods Fasting plasma glucose, insulin, leptin and lipoprotein concentrations, glucose and insulin responses to 75 g oral glucose tolerance test (OGTT) and insulin suppression tests were determined in 92 nondiabetic hypertensive patients (48 men and 44 women) and 92 age, BMI-matched controls.

Results FPL concentrations were higher in hypertensive men than in normotensive men (5.1 $\pm$ 0.5 versus 3.9 $\pm$ 0.4 ng/ml, P<0.02). FPL concentrations were not significantly different between hypertensive and normotensive women (11.8 $\pm$ 1.0 versus 10.9 $\pm$ 1.0 ng/ml, P=0.44). Although FPL concentrations showed good correlation with BMI, body fat, fasting plasma insulin concentrations, and insulin area to OGTT in both men and women (all P<0.001), it were related to steady state plasma glucose (SSPG) concentrations, a measure of insulin sensitivity by insulin suppression test, in men only (p<0.03). After adjustment for body fat amount, age and duration of hypertension, FPL levels were still significantly related to SSPG concentrations in men but not in women. FPL concentrations increased progressively across 3 different degrees of SSPG concentrations in men (P<0.01) but not in women (P=0.39).

Conclusion Higher FPL levels in hypertensive men but not in hypertensive women when compared with normotensive controls was demonstrated. These observations are consistent with the previous findings that plasma leptin is correlated with insulin sensitivity in men but not in women.

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Title: Difference regarding the relation between degree of obesity, leptin and parameters of the metabolic syndrome in diabetic and non-diabetic subjects.

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Aim: We investigate differences regarding the relation between leptin and several parameters of the metabolic syndrome in type 2 diabetic patients and non-diabetic subjects.

Methods: The study group consisted of 34 type 2 diabetic patients (Group A: 30 males and 4 females) and 19 non-diabetic subjects, as control group (Group B: 13 males and 6 females). BMI and WHR were determined. After an overnight fast, blood was drawn for the determination of total cholesterol, triglycerides, HDL (enzymatic method),apoA, apoB, apoE (immunoturbidimetric assay),fibrinogen, tp-A,PAI-I (EIA).We also measured plasma levels of insulin, proinsulin, leptin (RIA method) and HbA_1c. Insulin treated diabetic patients and patients with hepatic, thyroid and renal dysfunction and under hypolipidemic therapy were excluded from the study. Statistical analysis was performed using Mann-Whitney test regarding comparisons and Pearson test regarding correlation's. The level of significance was at p<0.05.

Results: The two groups were comparable according to age (54.79 ± 6.01 yr. vs 51.42 ± 6.53 yr., p:ns), BMI (28.2 ± 3.39 vs 26.73 ± 3.09,p:ns) and WHR (0.967 ± 0.075 vs 0.932 ± 0.076,p:ns).We observed that diabetic patients had higher plasma levels of LDL-cholesterol (129.57 ± 36.71 vs 106.63 ± 37.27, p=0.034), lower HDL (42.67 ± 11.39 vs 60.26 ± 19.49,p=0.003) and higher levels of PAI-I (60.47 ± 31.87 vs 43.45 ± 27.34,p=0.036).Additionally group A patients had higher proinsulin levels in comparison to group B (14.23 ± 11.77 vs 8.58 ± 4.2,p=0.036), while leptin did not differ significantly between the two groups (6.15 ± 4.61 vs 8.41 ± 6.72,p:ns). Regarding correlation's, BMI in diabetic patients showed significant correlation with apoB (r=0.41,p=0.031), leptin (r=0.418,p=0.013) and insulin (r=0.419,p=0.013),while in controls BMI correlated with plasma levels of insulin (r=0.57,p=0.009) and PAI-I (r=0.62,p=0.004). Additionally WHR showed significant positive correlation with triglycerides in the diabetic group (r=0.38,p=0.041).

Conclusion: No significant differences were observed regarding plasma leptin levels between diabetic patients and controls. In both groups BMI is strongly related with insulinaemia, while in diabetic patients BMI is also related to leptin.

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RELATION OF LEPTIN WITH METABOLIC PARAMETERS IN FEMALE PATIENTS WITH NON-INSULIN DEPENDENT DIABETES MELLITUS

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Aim: To evaluate the relation of leptin with metabolic control parameters and the effect of 14 days of diet or diet + oral antidiabetic therapy on leptin concentration in female patients with non-insulin dependent diabetes mellitus (NIDDM). **Materials and Methods:** 51 female patients with NIDDM are treated with diet alone or diet plus glipizide-GITS, or gliclazide or metformin for 14 days. Plasma leptin is measured in basal and post-treatment period along with the metabolic markers. 35 healthy female subjects formed the control group. **Results:** Leptin levels were similar both at baseline (15.9±7.4 vs 18.3±17.7, respectively) and after treatment (15.4±7.5 vs 18.3±17.7, respectively) in diabetic and control groups. NIDDM patients with basal fasting plasma glucose (FPG)<180mg/dL had significantly higher leptin levels than NIDDM patients with basal FPG >=180 mg/dL (19.6 ± 8.7 vs 13.65 ± 5.4, respectively; p<0.05). Patients with basal PPPG<250 mg/dL also showed higher leptin levels than those with basal postprandial plasma glucose (PPPG)>=250 mg/dL (20.2 ± 7.9 vs 12.9 ± 5.2, respectively; p<0.05). Mode of treatment did not influence leptin levels. Δ leptin showed a weak correlation with basal FPG (r=0.346; p<0.05), basal and post-treatment PPPG (r=0.335, p<0.05 and r=0.325, p<0.05, respectively) and a moderate correlation with post-treatment FPG levels (r=0.391, p<0.01) in patients with NIDDM. **Conclusions:** 1) Leptin levels are similar between female patints with NIDDM and healthy females; 2) improvement in FPG and PPPG levels do not influence leptin levels in short term, but; 3) change in leptin level by treatment is positively correlated to FPG and PPPG levels. We conclude that leptin level is not affected by the presence of NIDDM and by short term treatment with diet alone or diet plus different oral antidiabetics but directly related to metabolic control in female patients with NIDDM.

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EFFECT OF CENTRAL NEUROPEPTIDE Y AND LEPTIN ADMINISTRATION ON OBESITY AND DIABETES IN *PSAMMOMYS OBESUS*.

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Previous studies have demonstrated that intracerebroventricular (ICV) administration of leptin significantly reduces food intake, percent body fat and bodyweight in leptin sensitive *ob/ob* mice, while ICV neuropeptide Y (NPY) administration is associated with hyperphagia and the development of obesity in these mice. However, little is known about the effects of either ICV leptin or NPY administration in polygenic animal models. **Aims:** This study investigated the effects of chronic ICV leptin and NPY treatment in *Psammomys obesus*. **Material and methods:** *Psammomys obesus* is a polygenic animal model of obesity and diabetes. These rodents naturally develop a range of abnormalities when fed a diet of standard laboratory chow, which closely resemble metabolic changes seen in the development of obesity and diabetes in human populations. *Psammomys obesus* were fitted with an ICV cannula situated in the lateral ventricle at least 7 days prior to treatment, after which 15 µg/day of leptin, NPY or saline was administered for 7 days via Alzet pump. **Results:**

Group	Bodyweight (g)	%change Bodyweight	Estimated %body fat	%change Glucose	Cumulative Food Intake
NPY	197.7 ± 7.5	5.72±1.28*	4.65±0.17*	76.0±38.3*	108.7 ± 7.8
SALINE	207.3 ± 4.1	1.95±0.73	3.97±0.26	0.02±0.82	97.3 ± 5.8
LEPTIN	193.7 ± 5.3	-0.08±0.58*	3.08±0.28*	-0.67±4.73	89.0 ± 4.1

* p < 0.05 compared to saline control

Chronic ICV NPY administration significantly increased percent change in bodyweight and estimated percent body fat when compared to control animals. In addition, ICV NPY treatment was associated with the development of diabetes in susceptible animals. In comparison, ICV leptin administration significantly decreased percent change in bodyweight and estimated percent body fat. **Conclusion:** ICV administration of 15 µg/day NPY and leptin significantly altered energy balance and bodyweight regulation in *Psammomys obesus*. Interestingly, only minor changes in body fat accumulation were necessary to trigger diabetes development following NPY treatment. The role of NPY and disturbances in energy balance will be further examined in this polygenic animal model of type 2 diabetes.

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HYPOTHALAMIC OREXIN GENE EXPRESSION IN ZUCKER FATTY AND ZUCKER DIABETIC FATTY RATS

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Aims: Orexins may play an important role in control of energy balance. Here we studied orexin gene expression in two rat models of hyperphagia, namely Zucker fatty (ZF) and Zucker diabetic fatty (ZDF) rats and their respective lean controls (ZFL, ZDFL). **Materials and Methods:** Orexin gene mRNA was measured using Northern Blotting in hypothalamic blocks. Body weight and various plasma metabolic indices were also measured as shown below. **Results:**

	ZF	ZFL	ZDF	ZDFL
Body wt (g)	469 ± 11**	293 ± 6	409 ± 20	423 ± 12
glucose (mM)	11.1 ± 1.3	8.8 ± 0.5	23.9 ± 1.6**	5.32 ± 0.7
insulin (ng/ml)	196.4 ± 99.1*	23.2 ± 2.4	4.2 ± 0.4**	1.26 ± 0.1
leptin (ng/ml)	12.4 ± 0.3**	2.3 ± 0.4	22.6 ± 3.5**	10.0 ± 0.9
orexin mRNA	0.69 ± 0.06*	1.00 ± 0.10	1.03 ± 0.15	1.00 ± 0.14

**P<0.01, *P<0.05 compared with lean counterparts (Student's t-test)

Hypothalamic orexin mRNA levels were reduced in ZF rats, consistent with published work for *ob/ob* and *db/db* mice. By contrast, ZDF rats showed no change in orexin gene expression. Like *ob/ob* and *db/db* mice and ZF rats, ZDF rats show hyperphagia, hyperinsulinemia and a defective leptin system but do not exhibit excessive weight gain. A common factor for the three models with reduced orexin mRNA levels is marked obesity, suggesting that reduced orexin gene expression is an adaptive response to excessive weight gain. **Conclusion:** Our data suggest that the hypothalamic orexin system is responsive to energy status and that orexin gene expression is suppressed to oppose further weight gain in obesity.

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TISSUE-SPECIFIC PROCESSING OF COCAINE- AND AMPHETAMINE-REGULATED TRANSCRIPT PEPTIDES IN THE RAT.

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Cocaine- and amphetamine-regulated transcript (CART) is a recently discovered hypothalamic peptide regulated by leptin and with a potent appetite-suppressing activity. In the rat, the CART gene encodes for a peptide of 116 amino acid residues or a splice variant 13 residues longer. The predicted length of the prohormone is 89 amino acids. The CART prohormone contains several potential posttranslational processing sites in the form of mono- and dibasic sequences. **Aims**: Identification and characterisation of the natural occurring forms of CART peptides in the central nervous system as well as in the periphery. **Methods**: Frozen tissue from 18 female Wistar rats were used for a ethanol/HCl extraction. The extracts were passed over affinity columns containing the CART C-terminal specific monoclonal antibody Ca6-F4D4. The CART peptides were purified to homogeneity by reverse phase HPLC on a Vydac 214TP54 C4. The N-terminal amino acid sequence were determined by automated Edman degradation (Applied Biosystem Model 494 Sequencer) and mass spectrometry analysis were performed on a Voyager RP (Perseptive Biosystems). **Results**: In the present study we have isolated CART peptides from adrenal gland, hypothalamus, nucleus accumbens and pituitary gland of the rat and determined the peptide structures by using microsequencing and mass spectrometry. From the adrenal gland we isolated two long forms of CART (1-89 & 10-89), whereas only short form of CART (42-89 & 49-89) were isolated from the hypothalamus, nucleus accumbens and pituitary. Preliminary data of CART peptide expression in the GI-tract (stomach, duodenum & jejunum) indicate that the shorts forms are 3 times more abundant than the long forms. **Conclusion**: The proteolytic processing of the CART precursor was studied both in central and in peripheral tissues from the rat. The CART precursor was found to be processed differently in central and peripheral tissues. This tissue specific processing indicates that CART peptides may have different biological function in the periphery and in the central nervous system.

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Leptin II and Adipositas

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DIETARY PROGRAMMING OF INSULIN-LEPTIN INTERACTIONS

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Aims: Recent studies suggest a link may exist between poor early (fetal and postnatal) nutrition and development of insulin resistance in later life. Obesity is also a risk factor. The study assessed whether early protein restriction and/or high-fat (HF) feeding in adulthood modify the regulatory interactions between insulin and leptin. **Materials and Methods:** Pregnant dams were fed either 20% or 8% protein isocaloric diets during pregnancy and lactation and female offspring (termed control (C) and early-protein-restricted (EPR) groups respectively) were fed 20% protein diet from weaning. At 150 days of age, subgroups of C and EPR groups were fed a HF diet (C-HF and EPR-HF) and studied after 28 days. Plasma leptin responses were studied in the four groups in the postabsorptive state and after euglycaemic hyperinsulinaemia. **Results:** Plasma leptin levels were 32% lower in EPR compared with C offspring which may, in part, reflect lower body weights (by 13%) in the EPR group. Euglycaemic hyperinsulinaemia in vivo elevated plasma leptin levels in C and EPR groups by 1.3-fold (NS) and 2.2-fold (P<0.01) respectively. The effect of insulin to elevate plasma leptin levels was 2.8-fold greater, and leptin levels after insulin stimulation were 16% higher, in the EPR group. Differences between postabsorptive leptin levels in the C and EPR groups were no longer observed after 28 days of HF feeding. The leptin response to hyperinsulinemia was unaffected by HF feeding in the C group. In contrast, HF feeding abolished the leptin response to hyperinsulinaemia in the EPR group. As a consequence, plasma leptin levels after insulin stimulation were 39% lower in the EPR-HF compared with the EPR group. **Conclusions:** Leptin has an established role in signalling satiety. The present studies suggest that EPR, by programming enhanced leptin responsiveness to insulin, programmes satiety. Importantly, the subsequent imposition of a HF diet abolishes the programmed leptin response, and impairs leptin responsiveness to insulin. Thus, consumption of a HF diet after poor early nutrition would be predicted to increase the risk of obesity through dysregulation of appetite control.

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IMPACT OF DIETARY LONG-CHAIN OMEGA-3 FATTY ACIDS ON PLASMA LEPTIN LEVELS AND HEPATIC GLYCOGEN STORAGE

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Aims: Diets high in saturated fat impair insulin action, but replacement of a small proportion of saturated fat with long-chain ω-3 fatty acids (from fish oil) prevents insulin resistance. We assessed the impact of the latter manipulation on leptin levels in rats rendered insulin resistant by high-saturated-fat feeding. In view of emerging evidence that leptin enhances hepatic glycogen storage (HGS), we also evaluated the relationship between plasma leptin levels and HGS. **Materials and Methods:** Adult female rats were fed either standard diet (8% fat, 72% carbohydrate) or one of two isocaloric high-fat diets (47% fat, 33% carbohydrate) differing in fatty acid (FA) composition for 28 days. The first (SAT-fat) diet contained saturated fat as the major lipid (43% of total energy). The second (Omega-3) diet was identical to SAT-fat except that 7% of the dietary saturated FA was replaced with long-chain ω-3 FA. **Results:** Despite inducing insulin resistance, SAT-fat feeding did not alter plasma leptin levels in the absorptive state, whereas the Omega-3 diet significantly elevated (3.3 fold; P<0.001) plasma leptin levels. Paradoxically, hyperleptinaemia in the Omega-3 group was associated with reduced HGS (by 48%; P<0.001). Plasma insulin levels, moderately lower (26% vs. control, N.S.) in the SAT-fat group, were significantly suppressed (by 44% vs. control; P<0.05) by ω-3 FA supplementation. A significant positive linear correlation (r=0.97) existed between HGS and insulin levels in the three groups. **Conclusions:** The data demonstrate that dietary FA composition, rather than fat content, is the major determinant of the leptin response to dietary lipid. However, hyperleptinaemia in rats provided with a high-saturated fat diet enriched in long-chain ω-3 FA is not associated with enhanced HGS. This suggests that leptin's action to promote glycogen storage is synergistic with or dependent upon hyperinsulinaemia, or that liver glycogen storage responds to decreasing rather than to increasing leptin levels (i.e. a fall in leptin promotes glycogen loss).

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REGULATION OF THE 24h LEPTIN PROFILE IN MICE

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Aim. To establish the mechanism underlying the diurnal variation and the nocturnal peak in circulating leptin. **Materials and methods.** Blood was sampled every 2nd hour for 24 hours in fed as well as in fasted female and male mice of the NMRI strain for the determination of leptin, insulin and glucose. **Results.** *In the baseline samples* at 8 a.m., leptin correlated to body weight ($r=0.44$, $p<0.001$) and insulin ($r=0.39$, $p<0.001$), but was not different between the genders (6.2±0.3 ng/ml in females, n=115, vs. 6.4±0.5 ng/ml in males, n=80, body weight not different). In contrast, baseline insulin was higher in males than in females (576±47 vs. 275±21 pmol/l, $p<0.001$) as was baseline glucose (10.8±0.2 vs. 8.7±0.2 mmol/l, $p<0.001$). *In the fed state*, circulating leptin showed a diurnal variation in both genders; the increase was significant in samples taken at 2 p.m. and leptin peaked at 12 p.m. The leptin peak was higher in female than in male mice (3.5±0.3 vs. 1.3±0.3 ng/ml, $p<0.001$) in spite of exaggerated nocturnal increase in insulin in male mice (406±82 vs. 136±41 pmol/l, $p=0.004$). Although the nocturnal increases in leptin and insulin correlated to each other in both genders ($r=0.49$ in males, $r=0.34$ in females, $p<0.05$), the slope between increases in leptin and insulin was higher in females than in males (2.7±0.1 vs 1.7±0.1 ng/pmol, $p<0.001$). *In fasted animals*, circulating leptin did not display any nocturnal rise, but instead declined throughout the 24h as did circulating insulin. These declines were more pronounced in male than in female mice ($p<0.001$) and correlated to each other ($r=0.29$, $p=0.011$). The overall 24h plasma leptin significantly correlated to gender, body weight, insulin and glucose. **Conclusion.** Insulin governs the nocturnal leptin peak although the influence of insulin is markedly gender dependent.

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GLUCAGON-LIKE PEPTIDE-1 (GLP-1) AND LEPTIN SECRETION AFTER AN ORAL GLUCOSE LOAD IN OBESE PATIENTS.

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GLP-1 is mainly secreted in response to carbohydrate-rich meals, and probably contributes to postprandial inhibition of food intake. Leptin mRNA expression has been recently described in gastric epithelial cells, and the hypothesis of a gastric postprandial secretion of leptin has been formulated. Aim of this study is the assessment of the effect of oral glucose on leptin and GLP-1 secretion in obese patients. The study was performed in 10 obese nondiabetic male patients, aged (m±sd) 47.1±11.4 years, with a BMI of 34.7±3.5 kg/m^2. In order to avoid the interference of variations of glycaemia and insulinaemia on leptin and GLP-1 secretion, an oral glucose load (50 g) was administered 90' after the beginning of a euglycaemic hyperinsulinaemic clamp, and circulating leptin, GLP-1 (7-37), and GLP-1 (7-36) amide were measured at 0, 30, 60, and 90', while maintaiing plasma glucose at 5.5 mmol/l. Circulating GLP-1 (7-36) amide was (median [25°-75° percentile]) 128 [100-166.9] pg/ml at 0', 190 [171.2-236.8] at 30', 181.2 [133.7-295] at 60', and 176.2 [138.1-220.6] at 90' ($p<0.01$ at ANOVA; 30, 60, and 90' $p<0.01$ at Wilcoxon test *vs* 0'). GLP-1 (7-37) was 19.0 [6.4-26.3] pg/ml at 0', 22.1 [13.5-32.3] at 30', 19.1 [10.1-33.7] at 60', and 20 [14.4-33.2] at 90' ($p<0.05$ at ANOVA; 30' $p<0.05$ at Wilcoxon test *vs* 0'). Leptin was 13.6 [12-19] ng/ml at 0', 13.9 [12.6-20.1] at 30', 14.1 [12.6-21.9] at 60', and 14.6 [13.3-20.5] at 90' (p=NS). In conclusion, an oral glucose load in isoglycaemic isoinsulinaemic conditions determines a significant increase in GLP-1, but not leptin, secretion.

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ELEVATED PLASMA LEVELS OF α-MSH ARE CORRELATED WITH HYPERINSULINEMIA AND INSULIN RESISITANCE IN OBESE MEN

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Aims: The role of α-melanocyte-stimulating hormone (MSH) in obesity and in the levels of leptin has been well-documented. However, circulating level of α-MSH and its relationship with clinical parameters of obesity and glucose metabolism have not been as yet evaluated. In this study we evaluated the clinical significance of circulating levels of α-MSH in obese men. **Materials and Methods:** We measured the plasma levels of α-MSH in 12 obese (age: 38.0 ± 1.2 [mean ± SE] years, BMI: 28.7 ± 0.7 kg/m^2) and 12 non-obese (age: 40.0 ± 2.0 years, BMI: 22.3 ± 0.6 kg/m^2) men. The relationship of the plasma levels of α-MSH with the degree of obesity, body fat weight, the visceral and subcutaneous fat areas, insulin fasting levels, insulin resistance and with the serum levels of leptin and TNF-α was also evaluated. Circulating levels of α-MSH were measured by a commercial radioimmunoassay (RIA) kit. Body fat weight was measured by bioelectric impedance. Visceral and subcutaneous fat areas were measured by computed tomography (CT) and insulin resistance was assessed by the glucose infusion rate (GIR) during an euglycemic hyperinsulinemic clamp study. Serum leptin levels were determined by radioimmunoassay using human leptin RIA kit and TNF-α in serum samples was measured using a commercially available sandwich immunoassay kit. **Results:** In obese subjects, the plasma levels of α-MSH were significantly increased compared with those in non-obese subjects ($P < 0.05$); they were positively correlated with the BMI ($r = 0.584$, $P < 0.01$), insulin fasting levels ($r = 0.518$, $P < 0.02$) and with the visceral fat areas ($r = 0.760$, $P < 0.01$), but negatively correlated with GIR ($r = -0.591$, $P < 0.01$). Circulating levels of α-MSH were not significantly correlated with the body fat weight, subcutaneous fat areas and with the serum levels of leptin and TNF-α. **Conclusions:** The present study showed that circulating levels of α-MSH are increased in obesity and that they are positively correlated with obesity-associated hyperinsulinemia and insulin resistance.

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This abstract has been withdrawn

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COMPOSITION OF INSULIN-INDUCED BODY WEIGHT GAIN IN 72 DIABETIC PATIENTS STUDIED BY BIO-IMPEDANCE ANALYSIS.

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Aims:Although insulin is a well-known cause of body weight gain, it is not clear whether it is due to the accumulation of fat or lean mass. Materials and Methods: We performed a 3 months Body-Impedance Analysis follow-up in 72 diabetic patients in a wide range of insulin indications: insulin introduction in young inaugural IDDM (n=12), late-onset IDDM (n=12), NIDDM affected by intercurrent diseases (n=12) or microangiopathic complications (n=12), NIDDM with failure of oral antidiabetic agents (n=12), and insulin withdrawal in NIDDM (n=12). Results: In IDDM, insulin led to the most important weight gain (+3.0±1.0kg/30d in young inaugural, $p<0.05$ vs NIDDM; +2.0±0.9 in late-onset IDDM), but it was fat-free, with a major benefit on HbA1C (-7.3±1.1% young; -5.8±1.0 late-onset). NIDDM patients affected by intercurrent diseases or microangiopathic complications had a mild (both +0.5±0.3 kg/30d, $p<0.05$ vs IDDM), also fat-free weight gain, with a clear benefit on HbA1C (-5.2±1.1% interc. disease; -3.6±0.4 microangiop.). In NIDDM patients with failure of oral agents, HbA1C declined less (-2.7±0.6%,$p<0.01$ vs IDDM), weight gain was intermediar (+1kg/30d), but predominantly fat (+0.6±0.3 kg/30d, $p<0.05$ vs IDDM), mirrored by a predominant fat loss (-0.9±0.4 kg fat/30d from -1.3 kg weight/30d) in NIDDM patients whose insulin was stopped (without significant change in HbA1C). Both fat and lean mass contributed to insulin-induced body weight gain, but a significant negative relationship existed between their respective evolution in our patients ($r=-0.23$, $p<0.05$ by linear regression analysis between Δfat mass and Δlean mass). Conclusion: Insulin-induced body weight gain is not univocal: insulin restaures or protects lean mass in its less controversial indications, whereas it leads to fat accumulation in NIDDM with isolated failure of oral agents. BIA offers a simple method to precise the benefitial or detrimental effect of insulin in diabetic patients.

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REGULATION OF GLYCOGEN SYNTHESIS AND BREAKDOWN IN HUMAN OBESITY.

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Aims. Defect in glucose storage is the most important marker of insulin resistance. As the amount of glycogen stored depends not only on glycogen synthase (GS) but also on glycogen breakdown, the purpose of the work was to study the effect of obesity on the regulation of both GS and glycogen phosphorylase (GP). **Materials and methods.** 23 obese nondiabetic patients were compared with 15 lean control subjects. A euglycemic, hyperinsulinemic, clamp was performed in association with indirect calorimetry. Muscle needle biopsies were performed before and at the end of the 2-hr clamp for measurements of GS and GP activities and of glycogen concentration. **Results.** A strong positive correlation was observed during the clamp between glycogen concentration and GP activity in the obese group ($p=0.0001$; lean: p=NS) corresponding to stimulation of the enzyme by glycogen against limitation of the utilization of glucose from glycogen. GS activity was negatively correlated to glycogen concentration in the obese group ($p=0.037$; lean: p=NS) corresponding to inhibition of the enzyme by glycogen. Glucose oxidation during the clamp was lower in the obese than in the control group (75.2 ± 5.5 vs 105.0 ± 6.1 mg/m^2.min, $p=0.002$). The decrease in the activation of GS by insulin (ΔGS) was correlated to the decrease in glucose oxidation ($p=0.012$). **Conclusions.** The decrease in glucose storage in obesity is accompanied by a decrease in glucose oxidation. It is proposed that the decrease in muscle glucose oxidation indirectly leads to an inhibition of GS and glucose storage in obesity through alterations in glycogen mobilization. This mechanism, specific for glucose storage, does not exclude a defect in glucose transport.

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GLP and Glucagon I

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NEW HIGHLY SPECIFIC IMMUNOASSAYS FOR GLUCAGON-LIKE PEPTIDE 1 (GLP-1).

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Aims: Glucagon-like-peptide-1 (GLP-1) is present in plasma in minute amounts (pM range). Furthermore it is rapidly degraded to a number of degradation products. The degradation takes place both N- and C-terminally, but the most critical from a functional point of view is the N-terminal degradation. This study was undertaken to 1: demonstrate the specificity of two new GLP-1assays and 2: compare the profiles of GLP-1 induced after a meal measured in the two assays. **Materials and Methods:** Three monoclonal antibodies against GLP-1 were used: An N-terminal reacting antibody (MAb26.1), a C-terminal reacting antibody (GLP1F1) and an antibody less sensitive to C-terminal degradation reacting around position 26 (GLP1F5). Two two-site (sandwich) immunoassays were constructed with either GLP1F1 or GLP1F5 as catching antibody. In both assays MAb26.1 was used as detecting antibody. The following peptides were synthesized: GLP-1(7-37), (1-37), (8-36)amide, (9-37), (11-36)amide, (7-36)amide, (7-35), (7-34), (7-33), (7-38) and GLP-1(7-41) and used for demonstration of specificity. Human plasma samples from a number of healthy volunteers was collected before and after a meal. **Results:** The specificities of the assays were demonstrated using the different peptides. With GLP-1(7-37) as reference, both assays showed <0.1% crossreactivity towards both N-terminally prolonged and truncated forms. The assay with GLP1F1 showed, as expected, the greatest specificity C-terminally with <0.1% crossreactivity on all C-terminally truncated forms. The concentrations measured in plasma before and after a meal ranged from 1pM to 15pM. **Conclusion:** The previously reported levels of GLP-1 obtained with less specific assays are apparently overestimated. With these new assays, which are both more sensitive and more specific than other published assays for GLP-1, the biologically active form of GLP-1 can be monitored more accurately.

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GLP-1 SECRETION IN MORBID OBESITY AFTER VERTICAL BANDED GASTROPLASTY.

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Aims: morbidly obese patients show, frequently, glucose intolerance and insulin resistance, which tend to normalize after bariatric surgery; in this work we studied the possible implication of GLP-1, an incretin with antidiabetic properties, in that process. **Methods**: an oral glucose test (75 g) was performed before and six months after vertical banded gastroplasty (VBG), in 8 obese subjects (sex: 7F/1M; 42±4 years-old; BMI: 48.5±2.9 kg/m^2), previously informed consent given. Plasma glucose, GLP-1, insulin and glucagon were measured. **Results**: pre-VBG, 4 patients showed glucose intolerance. Six months post-VBG, the BMI was significantly decreased to 36.0±2.0 kg/m^2, and the glucose tolerance was normal in all subjects, being the paired glucose incremental area (0-180 min) significantly ($p<0.02$) reduced (-278±83 mM.min). No significant differences were observed in basal or glucose-induced insulin and glucagon responses, between pre- and post-VBG. Basal GLP-1 did not change after VBG, but the glucose-induced increment, from 15 to 90 min after glucose load, was about two-fold higher in post- than in pre-VBG, being statistically significant ($p<0.05$) the paired difference between the incremental area up to 180 min (36.4±13.8 ng.min.ml^{-1}). **Conclusions**: the higher oral glucose-induced GLP-1 secretion after reduction of obesity, with no changes in insulin or glucagon responses, indicates a GLP-1 effect on glucose disposal, either direct or through an increase in insulin sensitivity.

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GIP STIMULATES GLP-1 RELEASE IN CANINE JEJUNUM BY PARACRINE ACTION.

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Aims: The aim of this study was to evaluate a possible physiological role of our previously shown stimulation of GLP-1 release by GIP in cultured canine L-cells. **Materials and Methods:** Immunocytochemistry was used to establish the cell distribution of L-(GLP-1), K-(GIP) and D-cells (somatostatin) in the canine small intestine and distances between K- and L-cells, where they co-localize. Centrifugal elutriation of ileal single cell preparations were used for L-cell enriched short term cultures for secretion determinations. Time and GIP-concentration dependent studies of GLP-1 release were performed to evaluate possible down-regulation of the GIP-effect, as the disappearance of effect of GIP on insulin release is known. **Results:** K-cells were equally distributed in duodenum and jejunum. L-cells were found in duodenum (5%) jejunum (73%) and ileum (22%). D-cells were evenly distributed in the small intestine. The middle 50% of the small intestine contained 69% of the K-cells and 51% of the L-cells. More than 30% of these L-cells were adjacent to K-cells. Time dependent homologous down-regulation of the GIP effect (EC50: 0.7 ±.2 nM at 5 min and 17.2±5.2 at 120 min; P≤ .03) was found. **Conclusions:** Our results indicate that a paracrine action of GIP on GLP-1 release is very likely. This paracrine nature of action and homologous down regulation of effect may explain the lack of effect seen by exogenous GIP under certain physiological conditions.

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EFFECT OF GLP-1 ON LIVER AND FAT GPI/IPG SYSTEM IN DIABETIC STATES

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Aims: GLP-1 exerts insulin-like actions in rat liver, skeletal muscle and fat, being in liver and muscle not mediated by adenylyl cyclase activation. Although in lower magnitude than that in normal rats, the effect of GLP-1 upon glucose metabolic variables in liver and muscle of streptozotocin-induced non-insulin (STZ-NID) and insulin (STZ-ID) dependent diabetic models, is preserved. Mediation of an inositolphosphoglycan (IPG, second messenger in insulin action) on the effect of GLP-1 in BC3H1 and G-2 cells, and rat adipocytes and hepatocytes, was documented. Herein, we studied the effect of GLP-1, compared to that of insulin, on the kinetic of glycosylphosphatidylinositols (GPIs, precursors of IPGs), in adipocytes and hepatocytes from STZ-ID and STZ-NID rats. Methods: cells, prelabelled with *myo*-[^{3}H]inositol, were incubated without (control) and with GLP-1 or insulin; GPIs were analyzed in organic cell extracts by sequential TLC, autoradiography and ß-counting. Results: in **STZ-NID** rat **adipocytes**, 10^{-9} M GLP-1, as insulin, induced a rapid decrease of GPIs levels, followed by an increase over control at min 2 (0.5 min: -30±3% of control, $p<0.001$, n=7; 1 min: -16±3%, $p<0.01$, n=5; 2 min: +23±8%, $p<0.05$, n=7; 5 min: +7±10%, n=7; 10 min: +18±7%, $p<0.05$, n=7), as observed in normal animals; in **hepatocytes**, a delay in the hydrolysis of GPIs by GLP-1 was detected (0.5 min: +19±10%, n=18; 1 min: -19±7%, $p<0.01$, n=14; 2 min: -24±9%, $p<0.02$, n=15; 5 min: +45±11%, $p<0.01$, n=18; 10 min: +11±13%, n=18), while insulin did not exert any effect. In **STZ-ID** rats, neither GLP-1 nor insulin had any action upon IPGs generation in both cell types. Conclusions: GLP-1 receptor in fat and liver seems to be associated to GPI/IPG system, whose alteration could participate in the pathophysiology of diabetes; these data add support to the proposed benefit of GLP-1 in the therapy of type 2 diabetes mellitus.

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GASTRIC EMPTYING AND GLUCAGON-LIKE PEPTIDE 1 (7-36 AMIDE) RESPONSE TO A SOLID TEST MEAL IN TYPE 1 DIABETES.

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Efficient gastric emptying is dependent on the co-ordination of multiple neuroendocrine regulating mechanisms. Besides the plasma glucose excursions and the integrity of autonomic nerve function, Glucagon-like Peptide 1 (GLP-1) is a further physiologic modulator of gastric motility. In type 1 diabetes highly variable rates of gastric emptying may occur, but the pathogenesis of gastric abnormalities is unclear. **Aims:** to assess in type 1 diabetics the rate of gastric emptying in relation to: 1) autonomic nerve function degree, and 2) plasma GLP-1 secretion in response to a mixed meal. **Materials and Methods**: 16 type 1 diabetic patients with no symptoms of gastrointestinal dysfunction (mean age 40.5±14.4 yr, HbA1C=7.8±1.5%) and 9 healthy volunteers were studied. The autonomic nerve function assessment was evaluated by standardized cardiovascular reflex tests: 18.7% of the patients were affected by definite autonomic neuropathy, 50% presented only partial autonomic involvement, the remaining 31.3% was in the normal range. All subjects studied received a standard mixed breakfast (230 KCal: 60 % carbohydrates, 18 % proteins, 22 % lipids) containing labelled octanoic acid (1gr), as a marker for measuring the gastric emptying rate of solids (13-C octanoic acid breath test). Breath samples were taken every 15 min. for 4 hours and analyzed for 13-CO_2 by isotope ratio mass spectrometry. Blood samples were collected every 30 min. for 3 hours for plasma glucose, C-peptide, glucagon and GLP-1 determination. **Results:** in the control subjects meal ingestion induced a significant increase in plasma GLP-1 at 60 min. ($p<0.05$), returning towards fasting values in the following 2 hours. In contrast, in all diabetic patients examined, there was no increase in postprandial GLP-1 levels which were found even to decrease throughout the test ($p<0.05$). As a group, the diabetic patients showed a half time (T 50) of gastric emptying (100.5±59.8) that was not significantly different from the controls (88±28). No correlation was found between the T 50 value and the duration of diabetes, the fasting plasma glucose at the time of the study, the autonomic dysfunction degree, or GLP-1 response to the meal. Statistical evaluation of the results was performed by kruskall-Wallis analysis and Spearman correlation. **Conclusions**: 1) in type 1 diabetes gastric emptying is not thought to reflect the degree of autonomic function involvement 2) the impaired GLP-1 secretion observed in type 1 diabetic patients in response to food ingestion would not be able, in itself, to significantly affect the gastric emptying rate.

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GLUCAGON LIKE PEPTIDE-1 SIMILARLY AMPLIFIES INSULIN SECRETION IN LATE-ONSET TYPE 1 AND TYPE 2 DIABETES

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The impairment in insulin secretory responses to glucose (beta-cell function) in Late-Onset Autoimmune Diabetes (LADA) and Type 2 diabetes (T2) are presumed to have different aetiologies. GLP-1 may act by restoring subnormal intracellular cyclic AMP or as a pharmacological stimulus.

Aims: To compare the response of the beta cell function to GLP-1 infusion in LADA and T2. **Patients:** 12 LADA (islet cell antibody titre > 5 or glutamic acid decarboxylase antibody titre >20) and 12 matched T2 patients, mean (SD) age 62 (6) and 59 (6) years, BMI 32.4 (7.2) and 35.0 (6.9) $kg.m^{-2}$, in LADA and T2 respectively, with 2 sulphonylurea vs. 10 insulin treated in each group. **Methods:** Subjects had two step hyperglycaemic clamps (90 min 5 $mmol.l^{-1}$ and 90 min 13 $mmol.l^{-1}$) with infusions of 1.2 $pmol.kg^{-1}.min^{-1}$ GLP-1 or saline throughout . **Results:** The mean plasma C-peptide at the end of the 5 $mmol.l^{-1}$ clamp in LADA patients for Saline and GLP-1 were 0.25 and 0.62 $pmol.l^{-1}$ and at 13 $mmol.l^{-1}$ were 0.52 and 1.65 $pmol.l^{-1}$ with values in T 2 patients 0.20 and 0.48 and 0.61 and 1.97 $pmol.l^{-1}$ respectively. The plasma C-peptide responses at 5 $mmol.l^{-1}$ and 13 $mmol.l^{-1}$ during GLP-1 infusion correlated with those during saline infusion, Spearman Rank Rs = 0.91 and 0.96 in LADA and 0.92 and 0.85 in T2 respectively (all $p<0.0001$) with similar regression slopes and intercepts not significantly different from zero. GLP-1 stimulated secretion two fold and three fold at 5 and 13 $mmol.l^{-1}$, respectively. **Conclusions:** GLP-1 amplifies beta cell responses similarly in LADA and Type 2 diabetic patients.

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HYPOGLYCAEMIA-COUNTERREGULATION UNDER THE INFLUENCE OF EXOGENOUS GLUCAGON-LIKE PEPTIDE 1 (GLP1)

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Aims: GLP-1 and analogues are being evaluated as a new therapeutic principle for the treatment of Type 2-diabetes. GLP-1 suppresses glucagon secretion, which could lead to disturbances of hypoglycaemia counterregulation. **Materials and Methods:** Nine healthy volunteers with normal oral glucose tolerance received infusions of regular insulin (1 $mU \cdot kg^{-1} \cdot min^{-1}$) over 360 min on two occasions in the fasting state. Capillary glucose concentrations were clamped at plateaus of 78, 66, 54 and 42 mg/dl for 90 min each („stepwise hypoglycaemic clamp"); on one occasion GLP-1 (1.2 $pmol \cdot kg^{-1} \cdot min^{-1}$) was administered intravenously (steady-state concentration ≈ 125 pmol/l, on the other one NaCl as placebo. Glucagon, cortisol, growth hormone and catecholamines were determined, and autonomous or neuroglucopenic symptoms were assessed. Statistics: RM-ANOVA, t-tests. **Results:** At insulin concentrations of ≈ 45 mU/l glucose infusion rates were similar with and without GLP-1 ($p = 0.26$). Only during the euglycaemic plateau (78 mg/dl) GLP-1 suppressed glucagon concentrations (4.1 ± 0.4 vs. 6.5 ± 0.7 pmol/l, $p = 0.012$); at all hypoglycaemic plateaus glucagon increased similarly with GLP-1 or placebo, to maximum values > 20 pmol/l ($p = 0.97$). The other counterregulatory hormones and autonomous or neuroglucopenic symptom scores increased with decreasing glucose concentrations, but there were no significant differences comparing experiments with GLP-1 or placebo. **Conclusions:** The suppression of glucagon by GLP-1 does occur at euglycaemia, but not at hypoglycaemic plasma glucose concentrations (≤ 66 mg/dl). GLP-1 does not impair hypoglycaemia counterregulation.

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HYPERGLUCAGONEMIA DOES NOT INCREASE LIPOLYSIS IN ABDOMINAL ADIPOSE TISSUE BY MICRODIALYSIS

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Introduction: A possible role of glucagon on lipid metabolism still remains to be clarified. To determine whether glucagon induces lipolysis in adipose tissue healthy males were studied during a pituitary-pancreatic clamp. Lipolysis was evaluated with indwelling microdialysis catheters in abdominal adipose tissue. **Materials and methods:** Eight young males (23-34 years) participated in the study. Microdialysis catheters (CMA 60, Stockholm, Sweden), with a cutoff of 20 kDa, were placed subcutaneously in the abdominal adipose tissue. After perfusion for 1 hour with Ringer solution at a flowrate of 0.3 µl/min subjects were studied in 3 situations: 1. euglucagonemia (EU) (0.6 ng/kg/min); 2. hyperglucagonemia (HY) (1.8 ng/kg/min); 3. euglucagonemia (0.6 ng/kg/min), with a glucose infusion mimicking the glucose profile from the day of hyperglucagonemia (EU+HG). Somatostatin (450 µg/h) was infused to suppress endogenous hormone secretion and insulin (0.06 mU/kg/min) and GH (2 ng/kg/min) was infused for replacement. Sampling was done every 30 min for 7 h. Glycerol was analyzed on a CMA 600 apparatus (CMA, Stockholm, Sweden). **Results:** Baseline values of insulin, C-peptide, glucagon and GH were comparable in the 3 conditions. Baseline values of institial glycerol, plasma glycerol and serum free fatty acids were similar. There were no difference in the level of interstitial adipose glycerol (ANOVA: $p=0.3$) and likewise in the area under the curve (AUC) (97241±17979 (EU) vs 92221±11603 (HY) vs 79571±7684 (EU+HG) µM*390min, $p=0.6$). Plasma glycerol (ANOVA: $p=0.09$) and serum free fatty acids (ANOVA: $p=0.09$) were comparable. **Conclusion:** Hyperglucagonemia per se does not induce lipolysis in abdominal adipose tissue. Neither did hyperglycemia without hyperglucagonemia alter abdominal lipolysis.

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GLP and Glucagon II

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GLUCOSE-DEPENDENCE OF INSULINOTROPIC GLP-1 ACTIONS IN THE HYPOGLYCAEMIC RANGE: AN *IN VIVO* STUDY IN HEALTHY VOLUNTEERS

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Aims: In animal experiments, the insulinotropic actions of GLP-1 depend on elevated glucose concentrations. In human subjects, only rough estimates exist for this glucose-dependence. **Materials and Methods:** In nine healthy male volunteers with normal oral glucose, an exogenous (intravenous) infusion of human regular insulin (1 $mU \cdot kg^{-1} \cdot min^{-1}$) was maintained over 360 min on two occasions in the fasting state. Glucose concentrations were maintained at plateaus of 78, 66, 54 and 42 mg/dl for 90 min each (stepped hypoglycaemic clamp) by repeatedly adjusting glucose infusion rates according to the level of glycaemia determined every 5 min (glucose oxidase). On one occasion, GLP-1 (1.2 $pmol \cdot kg^{-1} \cdot min^{-1}$) was administered intravenously, leading to steady-state plasma concentrations of ≈ 125 pmol/l. On the other one, placebo (0.9 % NaCl/1 % HSA) was infused. Insulin and C-peptide were measured by immunoassays, insulin secretion rates were derived by deconvolution analysis (2-compartment model). Statistics: RM-ANOVA, t-tests. **Results:** At insulin concentrations of approximately 45 mU/l, basal glucose concentrations (90 ± 2 mg/dl) fell to 76 ± 2 mg/dl within 30 min. During this period, C-peptide concentrations increased with GLP-1 from basal 0.42 ± 0.10 to 1.13 ± 0.14 nmol/l at 30 min, but decreased thereafter, significant differences to placebo remaining until 240 min (glucose ≈ 54 mg/dl). However, when calculating insulin secretion rates, they increased with GLP-1 from basal 1.36 ± 0.31 to 4.86 ± 0.62 $pmol \cdot kg^{-1} \cdot min^{-1}$ after 30 min and sharply decreased thereafter even at 78 mg/dl glucose. No significant differences to placebo conditions were seen at glucose concentrations of 66 mg/dl or lower. **Conclusions:** Insulin secretion is stimulated by GLP-1 in humans at plasma glucose concentrations of 78 mg/dl (or greater), but not at all at lower levels of glycaemia. The insulinotropic action of GLP-1 is strictly glucose-dependent also *in vivo*.

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GLUCAGON-LIKE PEPTIDE-1(9-36)NH_2 NEITHER AFFECTS INSULIN RELEASE NOR ANTAGONISES THE INSULINOTROPIC EFFECT OF GLP-1(7-36)NH_2

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Aims: Glucagon-like peptide-1(7-36)NH_2 (GLP-1) is rapidly degraded *in vivo* by dipeptidyl peptidase IV (DPP IV), forming GLP-1(9-36)NH_2 which is an antagonist *in vitro*. Inhibition of DPP IV potentiates the insulinotropic effect of GLP-1 infused during an ivGTT. This effect could be due to increased levels of intact GLP-1 and/or to reduced levels of the potentially antagonistic metabolite. This study examined the contribution of these factors to the improved insulinotropic action seen after DPP IV inhibition. **Materials and Methods**: Valine-pyrrolidide (a specific DPP IV inhibitor; 300 µmol/kg iv) was administered to non-fasted anaesthetised pigs at –30 min. At 0 min, six animals received cross-over 40 min infusions of GLP-1(7-36)NH_2 (0.75 pmol/kg/min) alone or in combination with GLP-1(9-36)NH_2 (2.55 pmol/kg/min), and six had saline and GLP-1(9-36)NH_2. An ivGTT (0.2g/kg over 9 min) was given during min 21-30 of each infusion. Plasma GLP-1 was analysed by N-terminal RIA (intact GLP-1) and C-terminal RIA ("total" GLP-1). **Results**: Before inhibitor administration, basal levels of intact GLP-1 (19±3 pmol/l) were lower than total GLP-1 (51±9 pmol/l; $p < 0.01$). Valine-pyrrolidide reduced plasma DPP IV activity by >95% throughout the experiment and prevented GLP-1 degradation, raising the level of intact GLP-1 (52±10 pmol/l) to match the total levels (47±9 pmol/l). Infusion of GLP-1(7-36)NH_2 alone resulted in similar plateau values for intact and total peptide (intact, 97±8 vs total, 87±9 pmol/l). During co-infusion of GLP-1(7-36)NH_2 + (9-36)NH_2, similar plateau levels of intact peptide (102±14 pmol/l) were attained, but total GLP-1 levels were higher (207±17 pmol/l; $p < 0.005$). Plasma insulin responses to glucose were similar (AUC, GLP-1(7-36)NH_2, 3139±639 vs GLP-1(7-36)NH_2 + (9-36)NH_2, 2762±385 pmol/l*40 min), but were higher than in the saline treated group (AUC 1443±520 pmol/l*40 min; $p < 0.05$). GLP-1(9-36)NH_2 alone did not affect insulin secretion (AUC 1180±552 pmol/l*40 min; *ns* vs saline). **Conclusions**: These results indicate that GLP-1(9-36)NH_2 itself neither affects insulin secretion nor antagonises the insulinotropic effect of GLP-1(7-36)NH_2, suggesting that the increased insulin secretion previously seen after DPP IV inhibition is due to elevated levels of intact GLP-1 rather than removal of antagonism.

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DOWN REGULATION OF THE GLP-1 RECEPTOR CAUSED BY GLP-1 IS ASSOCIATED TO INCREASED RECEPTOR INTERNALIZATION.

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The glucagon-like peptide-1, GLP-1, has a well documented glucose potentiating effect on insulin secretion from beta-cell lines. We have previously shown that long term exposure of the beta cell line INS-1 to high glucose down regulates the glucose mediated insulin release. Exendin (9-39) is a GLP-1 receptor antagonist that binds to the receptor with an affinity identical to that of GLP-1 thereby blocking the stimulatory effect. In contrast to GLP-1, exendin does not cause internalization of the receptor. **Aims**: The aim of the present study was to elucidate a)whether GLP-1 can counteract the glucose mediated down regulation, b) if this effect is transient or persistent during long term exposure of the peptide itself, and c) how these changes are related to the exposure of the GLP-1 receptor on the beta cell. **Materials and Methods**: Studies were performed on the insulin secreting cell lines INS-1 and betaTC-3 cultured at 16.7 mM glucose with and without addition of either $10^{-10}/10^{-8}$ M GLP-1 or $10^{-10}/10^{-8}$ M exendin. BetaTC-3 cells were used for competitive binding assays and INS-1 cells for insulin secretion determination. Students unpaired t-test was used for statistical comparisons. **Results**: After 3 days exposure to 10^{-10} M GLP-1, the maximal binding was reduced by 55% compared to the binding after 3 hours of exposure ($p<0.001$). In contrast, the displacement of GLP-1 by exendin was not altered. In INS-1 cells the corresponding insulin output declined by 28% after 3 days exposure ($p<0.005$). As expected the insulin output in cells exposed 3 days to 16.7 mM glucose was reduced to 53% compared to cells exposed to 6.6 mM glucose ($p<0.001$). Interestingly, this down regulation was totally abolished by addition of 10^{-10} GLP-1. Exendin did not have any secretory capacity itself and counteracted the GLP-1 induced effect on the insulin output. **Conclusions:** Long term exposure to GLP-1 counteracts the glucose induced decrease in insulin output in INS-1 cells inspite of the down regulation of the surface exposed amount of GLP-1 receptor. The contemporary binding of exendin is unaltered pointing to an increase in GLP-1 receptor internalization caused by the GLP-1 peptide.

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DERIVATIVES OF GLUCAGON-LIKE PEPTIDE-1 SUITABLE FOR ONCE DAILY ADMINISTRATION.

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GLP-1 has been shown to be able to lower blood glucose effectively in type 2 diabetes. GLP-1 releases insulin in a glucose-dependent manner, decreases glucagon secretion, inhibits gastric emptying and has also been shown to decrease appetite. However, native GLP-1 is rapidly degraded and cleared from plasma making it difficult to use therapeutically. In the present abstract, we describe derivatives of GLP-1 which are both potent and metabolically stable and possess pharmacokinetic properties suitable for once daily administration.

Potency of compounds were determined using a functional assay employing the cloned human GLP-1 receptor. The relative *in vitro* metabolic stability was determined by mass spectrometry before and after incubation in human plasma and DPP-IV. Pharmacokinetic properties were determined after subcutaneous administration to pigs. Plasma concentrations were determined by use of a radioimmuno assay employing an antibody specific for the N-terminus of native GLP-1 and of the GLP-1 derivatives.

All compounds showed a markedly improved *in vitro* metabolic stability compared to GLP-1. The potency and plasma half-lives are listed in the table below. The most potent compound was Arg34Lys26-(N-ε-(γ-Glu(N-α-tetradecanoyl))) with an EC_{50} of 22 pM. The most protracted compound was Arg34Lys26-(N-ε-(γ-Glu(N-α-hexadecanoyl))) with a plasma half-life of 14h. All compounds were shown to be potent and sufficiently protracted to make once daily administration feasible.

Parent peptide (derivatized on N-ε-Lys)	Substi -tuent	Potency (EC_{50}, pM)	Plasma $t_{1/2}$ (h)
GLP-1		55 ± 19	1.2
Lys26 Arg34(7-37)	2	22 ± 7.1	9
Lys26 Arg34(7-37)	1	61 ± 7.1	14
Lys34 Arg26(7-37)	1	120 ± 26	13
Lys36 Arg26,34(7-36)	1	37 ± 2.1	13
Lys^{38}Gly^{8}Glu^{37}Arg^{26,34}(7-38)	1	140 ± 7.1	11

1 = γ-Glu-(N-α-hexadecanoyl), 2=γ-Glu-(N-α-tetradecanoyl).

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IMPROVED STABILITY AND GLYCAEMIC CONTROL WITH HIS^7-GLUCITOL GLUCAGON-LIKE PEPTIDE-1 IN NORMAL AND DIABETIC RODENTS

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This study examined the actions of glucagon-like peptide-1(7-36)amide (tGLP-1) and the N-terminally modified analogue His^7-glucitol tGLP-1 on glycaemic control in normal Wistar rats and obese diabetic ob/ob mice. Unlike native tGLP-1, His^7-glucitol tGLP-1 is resistant to the proteolytic actions of plasma dipeptidyl-peptidase (DPP) IV *in vitro*. Also His^7-glucitol tGLP-1 showed much greater *in vivo* resistance to degradation (92% intact) compared to tGLP-1 (27% intact) in plasma 10 min after i.p. administration to fasted (18 h) rats. Acute effects of tGLP-1 and His^7-glucitol tGLP-1 on glucose homeostasis were examined in rats (250-300g, n=6) following i.p. injection of each peptide (12 nmol/kg) together with glucose (18 mmol/kg). The area under the curve (AUC, 0-60 min, mean±SEM) for plasma glucose for controls (glucose alone, 691±35 mmol.l^{-1}.min) was significantly reduced after administration of tGLP-1 and His^7-glucitol tGLP-1 (36% and 49% less; AUC 440±40 and 353±31 mmol.l^{-1}.min, respectively; $p<0.01$, Students t-test). Acute effects of both peptides (12 nmol/kg) were also tested in fasted (18 h) obese diabetic ob/ob mice (20-25 weeks old, 75-100g, n=8). The AUC for glucose (0 to 60 min) in controls (836±130 ng.ml^{-1}.min) was significantly higher ($p<0.01$) than after tGLP-1 (526±85 ng.ml^{-1}.min) or His^7-glucitol tGLP-1 (575±66 ng.ml^{-1}.min). Following His^7-glucitol tGLP-1 injection, peak insulin responses at 30 and 60 min, were significantly greater (46.6±5.6 and 31.0±3.0 ng/ml, $p<0.05$-$p<0.01$) than for native tGLP-1 (38.5±5.4 and 24.2±3.3 ng/ml, respectively). Furthermore, higher AUC for plasma insulin concentrations were recorded for mice treated with His^7-glucitol tGLP-1 (849±141 ng.ml^{-1}.min) compared to glucose controls (207±103 ng.ml^{-1}.min, $p<0.01$) but not tGLP-1 (561±194 ng.ml^{-1}.min). A similar pattern of insulin response was observed in ob/ob mice given peptides (12 nmol/kg) in conjunction with feeding (30 min pre-injection). In conclusion, His^7-glucitol tGLP-1 is resistant to DDP IV *in vivo* and has potent glucose lowering and insulinotropic actions in normal and diabetic rodents.

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CHARACTERIZATION OF MUSCARINIC RECEPTOR SUBTYPES ON THE GLUTAG MOUSE ENTEROENDOCRINE CELL LINE

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Glucagon-like peptide-1 (GLP-1) is an enteroendocrine peptide that accounts for up to 60% of the pancreatic insulin released after an oral glucose load. A wide range of endogenous and exogenous secretagogues cause GLP-1 release from L-cells, which are mainly located in the small intestine. The effects of neurotransmitters on L-cells are still poorly understood. Cholinergic effectors stimulated GLP-1 secretion in an isolated vasculary perfused rat ileum preparation. Administration of acetylcholine to hypoglycemic dogs resulted in an elevation of plasma total immunoreactive glucagon. In humans, a clinical study also suggested the involvement of muscarinic receptors in GLP-1 secretion because atropine reduced GLP-1 levels in healthy subjects. In the first study we investigated the existence of muscarinic receptors on L-cells using a permanent mouse enteroendocrine cell line (GLUTag) and immunofluorescence labeling. FITC-labeled antibody M35 is directed against muscarinic receptor protein and does not discriminate between the five presently known subtypes. M35 labeling of GLUTag cells resulted in a positive signal on the plasma membrane of every cell. Fluorescence intensity was equally distributed throughout the preparation. On a functional level, we investigated the coupling between muscarinic receptors and GLP-1 release in a second study. The cholinergic agonist, bethanechol, stimulated GLP-1 secretion in a dose-dependent fashion (by 1.7 ± 0.3 fold at 500μM; n=4, $P<0.05$). Bethanechol-induced secretion could be blocked by atropine (at 1μM; n=4, $P<0.05$) confirming a direct involvement of muscarinic receptors in GLP-1 release. In order to characterize the muscarinic receptor subtypes expressed in GLUTag cells, RT-PCR was performed. In these studies expression of M_2, M_3 and M_4 receptor subtypes was observed. In conclusion, the present studies demonstrate the existence of muscarinic receptors on GLUTag cells and a functional coupling between these receptors and GLP-1 secretion. The individual roles of the different muscarinic receptor subtypes in GLP-1 release are currently under investigation.

GLUTag cells were obtained from D.Drucker, 1149336 Ontario Inc./Univ of Toronto

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ROLE OF PHOSPHATIDYLINOSITOL 3-KINASE IN INSULIN-INDUCED INHIBITION OF GLUCAGON SECRETION FROM IN-R1-G9 CELLS

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Aim: Intracellular mechanism by which insulin inhibits glucagon secretion has remained to be elucidated. We investigated using wortmannin, an inhibitor of phosphatidylinositol 3-kinase (PI3-kinase), whether the inhibitory effect of insulin on glucagon secretion was mediated through PI3-kinase pathway in In-R1-G9 cells, a pancreatic alpha cell line. **Materials and Methods:** Western blot analysis and PI3-kinase assay were performed to investigate whether insulin stimulate the phosphorylation of insulin receptor substrate-1 (IRS-1) and activate PI3-kinase. Static incubation study, Northern blot analysis and perifusion study were also performed using insulin (10^{-7} mol/l) and wortmannin (10^{-7} mol/l). The effects of insulin and wortmannin on subcellular localization of PI3-kinase were studied by Western blot analysis. **Results:** Insulin stimulated phosphorylation of IRS-1 and activate PI3-kinase, and wortmannin almost completely suppressed insulin stimulated PI3-kinase activity. In static incubation, insulin significantly ($p < 0.05$) inhibited the glucagon secretions at 2, 6, and 12h, which was completely abolished by pretreatment of the cells with wortmannin. In perifusion study, insulin significantly suppressed the glucagon secretion after 10 min, which was blocked by wortmannin. The glucagon mRNA was also decreased by insulin at 6 h (70 %) and 12 h (65%) but not at 2h. Wortmannin abolished the decrease of glucagon mRNA. Insulin increased the amount of 85 kDa subunit of PI3-kinase in plasma membrane fraction (PM), while reciprocal decrease of the kinase was observed in cytosol fraction (CY), and insulin also increased PI3-kinase activity in PM, but not in CY. **Conclusion:** Insulin could suppress the glucagon secretion by dual inhibition of glucagon release and glucagon gene expression both of which were mediated by the activation of PI3-kinase. The recruitment and activation of PI3-kinase in plasma membrane might be relevant in part to the insulin-induced inhibition of glucagon release.

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Requirement of a signal generated by nutrient metabolism for elicitation of insulinotropic effect of GLP-1

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The incretin hormone glucagon-like peptide-1 (7-36)amide (GLP-1) elicits strong insulinotropic effects in pancreas. To study the mechansims of action mediating GLP-1-induced hormone release, GLP-1 or CCK were administered to perfused rat pancreas (GLP-1 1 nM; CCK 1 nM; arginine 20 mM), isolated rat islets (GLP-1 100 nM), and in vivo in mice (GLP-1 32 nM/kg; arginine 25 mg/kg). In the isolated perfused rat pancreas, GLP-1 strongly potentiated insulin ($p<0.001$) and somatostatin release ($p<0.001$) in the presence of 16.7 mM glucose but failed to enhance 20 mM arginine-induced insulin or somatostatin release. Arginine-induced glucagon release was, however, promptly inhibited by GLP-1 ($p<0.02$). GLP-1 similarly enhanced glucose- but not arginine-induced insulin responses *in vivo*. Arginine-induced insulin release was enhanced both *in vivo* and *in vitro* by 1nM CCK ($p<0.001$) and its combination with GLP-1 did not further enhance the responses. To investigate whether cAMP generation modulates these hormonal action, GLP-1-induced cAMP generation and IBMX-induced insulin release were studied. GLP-1 (100 nM) failed to increase cAMP levels at either 3.3 or 16.7 mM glucose in isolated rat islets. IBMX (0.5 mM), in contrast to GLP-1, potentiated both glucose- and arginine-induced insulin release both in isolated rat islets and *in vivo* in mice. In conclusion, CCK and IBMX enhance the insulinotropic effects of both glucose and arginine both *in vivo* and *in vitro* while GLP-1 only enhances glucose responses . This finding taken together with the failure of GLP-1 to increase islet cAMP levels indicate that GLP-1 stimulates glucose-induced insulin release by rather influencing nutrtient metabolism than by generation of cAMP.

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C-Peptide Growth Factors and Growth Hormones

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PROINSULIN C-PEPTIDE DOES NOT BIND TO LIPID VESICLES

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Aims: Proinsulin C-peptide has been shown to improve renal function, ameliorate autonomic and sensory nerve dysfunction and stimulate blood flow and oxygen uptake in skeletal muscle in type I diabetes patients. It has been suggested that C-peptide interacts with a G-protein coupled membrane receptor, activating Ca^{2+} dependent intracellular signalling pathways, stimulating Na^+K^+ATPase and eNOS. Alternatively, C-peptide may elicit its effects independently of binding to a specific receptor by entering membrane lipid bilayers resulting in the formation of cation selective channels and alterations of intracellular ion homeostasis. To test the latter hypothesis, we examined whether C-peptide binds to lipid vesicles.
Materials and Methods: For structural studies, circular dichroism (CD) spectra (40 microM human C-peptide, 184-260 nm, 20 nm/min, 1-2 data points/nm, T=22 °C) of human proinsulin C-peptide in physiological salt solutions or in the presence of neutral or anionic phospholipid vesicles and micelles (0.5 mg/ml lipid in 25 mM sodium phosphate buffer) at different pH values have been recorded. In order to study physical association in solution, phospholipid vesicles/C-peptide mixtures were subjected to size exclusion chromatography. For this purpose a Superdex 200 HR 10/30 column, elution with 20 mM sodium phosphate buffer, 100 mM NaCl at a flow of 0.5 ml/min, were employed.
Results: If C-peptide is inserted into a membrane, the peptide structure is likely to be altered, compared to when it is freely moving in an aqueous solution. Likewise, C-peptide is expected to co-migrate with lipid vesicles upon size exclusion chromatography. In contrast, CD spectroscopy showed that proinsulin C-peptide was unstructured in aqueous solution and did not change its secondary structure in the presence of artificial lipid membranes, independent of vesicle charge. Furthermore, C-peptide and lipid vesicles did not co-migrate upon size exclusion chromatography, but were separated by approximately 40% of one bed volume.
Conclusion: Our data do not support a mechanism of action whereby C-peptide enters lipid membranes and forms ion channels.

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SPECIFIC BINDING OF PROINSULIN C-PEPTIDE TO HUMAN CELL MEMBRANES

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Aims. A series of recent reports have demonstrated beneficial effects of proinsulin C-peptide in the diabetic state, involving improvement of kidney and nerve function. To examine the background to these effects binding of C-peptide to cell membranes has been examined.
Materials and Methods. Measurements of ligand-membrane interactions at single molecule detection sensitivity in 0.2 fL confocal volume elements were carried out using fluorescence correlation spectroscopy.
Results. The findings show how specific binding of fluorescently labeled C-peptide to several human cell types. Full saturation of the C-peptide binding to the cell surface is obtained at low nanomolar concentrations. Scatchard analysis of binding to renal tubular cells indicates the existence of two binding processes with K_{ass} of $3.3 \cdot 10^9$ M^{-1} and $8 \cdot 10^9$ M^{-1}, respectively. Addition of excess unlabeled C-peptide is accompanied by competitive displacement, yielding a dissociation rate constant of $4.5 \cdot 10^{-4}$ s^{-1}. The data indicate the existence of a membrane-bound C-peptide specific receptor with a density of ~75 receptors/μm^2 in renal tubular cells and a lower density in human fibroblast and endothelial cells. The C-terminal pentapeptide displaces C-peptide bound to cell membranes, indicating that the binding occurs at this segment of the molecule. Non-native D-C-peptide and a randomly scrambled C-peptide do not compete for binding with the labeled C-peptide, nor were cross-reactions observed with insulin, IGF-I, IGF-II, or proinsulin. Modification of the receptor-coupled G-protein by pertussis toxin abolishes the binding.
Conclusion. C-peptide binds to specific receptors on human cell membranes, thus providing a molecular basis for the peptide's biological effects.

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THE EFFECT OF C-PEPTIDE ON CYCLIC GMP AND ERYTHROCYTE Na^+K^+APTase ACTIVITY IN DIABETES TYPE 1

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Aim: The study was undertaken to evaluate the influence of C-peptide on erythrocyte Na^+K^+ATPase and endothelial nitric oxide synthase (eNOS) activities in type I diabetes patients. **Materials and Methods:** Ten type I diabetes patients age 35 ± 2 yrs, diabetes duration 3-22 yrs, HbA1c 7.2 ± 0.5% received intravenous infusions of human C-peptide or saline on two different occasions in a double blind randomized study design. C-peptide was infused at rates of 3 pmol/kg/min for 60 min and then at 10 pmol/kg/min for 60 min. Blood samples (venous) were collected in the basal state and after 60 and 120 min for determinations of plasma cGMP (radioimmunoassay) and erythrocyte membrane Na^+K^+APTase activity (ATP-induced release of inorganic phosphate in the presence or absence of ouabain). **Results:** Basal plasma C-peptide 0.02 ± 0.02 nmol/l increased to 1.3 ± 0.1 nmol/l during the first infusion period and to 3.5 ± 0.3 nmol/l during the second. Plasma concentrations of cGMP were 5.5 ± 0.6 nmol/l in the basal state and rose to 6.8 ± 0.9 nmol/l during C-peptide infusion ($P<0.05$). Erythrocyte Na^+K^+APTase activity increased from 140 ± 29 nmol Pi/mg/h in the basal state to 287 ± 5 nmol Pi/mg/h during C-peptide infusion ($P<0.01$). There was a significant linear relationship between the plasma C-peptide levels and erythrocyte Na^+K^+APTase activity during the C-peptide infusion ($r=0.46$, $P<0.01$). No significant changes in plasma cGMP or Na^+K^+APTase activity were observed during the saline infusion study. **Conclusion:** The data demonstrate a direct influence of C-peptide on erythrocyte Na^+K^+APTase activity and provide indirect evidence that C-peptide administration also activates eNOS in type I diabetic patients.

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INSULIN-LIKE GROWTH FACTOR-I LEVELS ARE LOW AND NOT CORRELATED TO HBAIc IN ADULTS WITH TYPE 1 DIABETES.

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Aim: To study the plasma concentrations of insulin-like growth factor-I (IGF-I) in an adult population with type 1 diabetes in comparison to population-based reference values. Furthermore to study the influence of glycemic control, insulin dose, body composition and gender on the concentration of circulating IGF-I in type 1 diabetes.
Materials and methods: Blood sampling were consecutively collected in 80 men and 55 women 20-59 years old with diabetes type 1 and with over 6 years diabetes duration (range 6-51 years). A reference population of 80 men and 83 women 20-59 years old were randomly selected from the population registry. IGF-I was measured with radio-immuno-assay after acid-ethanol extraction.
Results: Mean values of IGF-I (± SD) for all ages and both genders were 146 (± 66) micrograms/L in diabetic patients and 237 (± 83) micrograms/L in control subjects, $P<0.001$. IGF-I declined with age in both diabetic patients and control subjects. When dividing after gender and age the difference in IGF-I levels between the two populations remained highly significant in all decades except in women 50-59 years. No correlation was found between glycemic control measured as HBA1c and IGF-I. The insulin dose corrected for body weight showed a positive correlation with IGF-1 in the male diabetes patients, $P<0.03$, but not for the female patients, $P=0,15$.
Conclusions: IGF-I levels in plasma are low in patients with type 1 diabetes in comparison to an age and sex matched reference population. The low IGF-I levels in plasma are not explained by poor glycemic control.

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DOSE DEPENDENT EFFECT OF INSULIN ADMINISTRATION ON IGFs & IGF-BPs SERUM LEVELS IN HEALTHY PEOPLE AT A POSTABSORTIVE PHASE

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Aims: To determine the dose-effect of insulin on circulating levels and splanchnic output of IGF-I, IGF-II, IGFBP-I, IGFBP-II and IGFBP-III in healthy human subjects. **Materials and Methods:** We investigated 12 each of lean (BMI=18-27kg/m^2 healthy men and women (Total=24, age=21-38 yr.) in the postabsorptive state. Following a three days of weight maintaining diet the subjects were studied after an overnight fast. After collecting two baseline blood samples from femoral artery and hepatic vein the subjects were randomly infused with either insulin at dosages of 0.25, 0.5, 1.0 um/ kg / min or saline for 150 min while maintaining euglycemia. Additional blood samples were collected at 140 and 150 min through the infusion and blood flow was measured by indicator dye dilution technique. Hormonal and binding protein levels were measured by radioimmuno assay. **Results:** Insulin decreased arterial and hepatic venous levels of IGF-I and IGFBP-I in a dose related manner ($P<0.01$) (eg; IGF-I decreased in artery from 290±34 to 264±30 ng/ml, $P=0.033$ at 0.5 mU/kg/min of insulin whereas during saline infusion there was no change. Insulin at 0.5mU/kg/min decreased IGFBP-I from 50±19 to 26±7 ng/ml while saline increased it from 58±13 to 101±17, $P=0.015$). Splanchnic output of IGFBP-I decreased in a dose-related manner ($P<0.01$) with the maximal effect at 0.5mU/kg/min of insulin infusion ((86±8% decline). No significant insulin effect was observed on circulating arterial or hepatic venous levels or splanchnic output of IGF-II, IGFBP-II and IGFBP-III. **Conclusion:** Insulin decreases IGF-I levels with no demonstrable effect on its splanchnic output. Insulin acutely decreases IGFBP-I output from splanchnic bed causing a decrease in circulating IGFBP-I levels.

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DETERIORATION IN GLUCOSE TOLERANCE FOLLOWING LONG TERM GROWTH HORMONE REPLACEMENT THERAPY.

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Introduction: Short time growth hormone (GH) replacement therapy in adults with pituitary deficiency leads to a deterioration of the insulin sensitivity without a compensatory increment in beta-cell function.
Aim: The aim of the present study was to evaluate the long term metabolic effects of GH given in a mean dose of 1.6 IU/day during a 30 months treatment period in adults with GH deficiency.
Materials and Methods: 11 (8 males and 3 females) GH deficient patients age 37.6 ± 9.9 (mean ±SD) years, BMI 27.1 ± 5.3 kg/m^2 participated. Glucose metabolism was evaluated by an oral (OGTT) and an intraveneously (FSIGT) glucose tolerance test and the body composition was estimated by a dual-energy X-ray absorptiometry (DXA) whole body scanning before and after treatment.
Results: GH treatment induced persistent favourable changes in body composition with a significant increase in lean body mass of 4.5 kg ($p<0.001$) and a reduction of the fat mass with 3.2 kg ($p<0.002$). Data from the OGTT showed a significant deterioration in glucose tolerance with an increase in the 2hr blood glucose (BG) value from 5.4 ± 1.6 to 7.4 ± 1.4 mmol/l ($p< 0.008$). Corresponding the incremental area under the curve $AUC_{glucose}$ ($p<0.003$), $AUC_{insulin}$ (0.005) and $AUC_{C\text{-}peptide}$ ($p< 0.0.003$) increased significantly. Four patients developed impaired glucose tolerance. Also fasting insulin level increased significantly from 30.2 ± 9.3 pmol/l to 55.5 ± 20.8 pmol/l, ($p< 0.003$) whereas fasting BG only increased from 5.0 ± 0.4 mmol/l to 5.16 ± 0.5 mmol/l (NS). The insulin sensitivity index (SI) decreased from 1.22 ± 0.7 10^{-4}x (min x pmol/l)$^{-1}$ to 0.95 ± 0.39 10^{-4}x (min x pmol/l)$^{-1}$ (NS). The non insulin dependent glucose uptake (glucose effectiveness S_G) was 0.0198 ± 0.004 min^{-1} and 0.022 ± 0.003 min^{-1} respectively (NS).
Conclusion: Long term GH replacement therapy has a significant diabetogenic effect evaluated by an OGTT with a significant increment in 2 hr BG value despite a significant increment in insulin secretion.

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INSULIN, OBESITY, GH, AND IGF-1 IN CHILDREN AFTER SURGICAL REMOVAL OF CRANIOPHARYNGIOMA.

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44 children (29 boys, 15 girls) with craniopharyngioma (CF) aged 3.6 to 18.9 yrs (11.6±3.1) have been analysed after surgical removal of CF. 24 patients had intrasellar tumour (IS), and 20 suprasellar (SS). Time after surgery was 2.6±2.8 (0.5 to 10.8) yrs. 19/20 patients with SS CF developed obesity after surgery: BMI in this group was significantly higher, than in patients with IS CF (25.0±5.4 vs 17.2±2.0, $p<0.001$). Obesity was accompanied by remarkable hyperinsulinemia: BMI significantly correlated with lgAUC of insulin (INS) secretion after oral glucose load (INSAUC) ($r=0.63$, $p<0.0001$), INSAUC in patients with SS CF was significantly ($p=0.001$) higher than in patients with IS CF (8667±5032 vs 3295±2555 mU/l·min). All patients had severe GH deficiency: peak of GH after clonidine test was less than 5 ng/ml, 39/44 children had GH less than 1.0 ng/ml after stimulation. 8/22 children had serum IGF1 value above 5th centile for age; IGF1SDS significantly correlated with lgINSAUC ($r=0,39$, $p=0.035$) and IGF1SDS was significantly greater in patients with SS CF than in patients with IS CF –2.0±1.1 vs –3.1±0.4, $p=0.004$). 15/25 children had height velocity above 5th centile for age, and 6/25 children had height velocity greater than 95th centile for age despite of GH deficiency. This phenomen was usually observed during first 12-18 months after surgery. Height velocity significantly correlayed with lgINSAUC ($r=0.352$, $p=0.008$) and with time after surgery ($r=-0.40$, $p=0.03$) but not with GH and IGF1 levels.

In conclusion: children with suprasellar craniopharyngioma develop obesity and hyperinsulinemia after surgical removal of the tumour due to hypothalamic lesion. It may cause normal IGF1 level and increased height velocity despite of GH deficiency.

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GROWTH HORMONE RESPONSE TO GHRP-6 IN NIDDM PATIENTS

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Growth hormone (GH) secretion is decreased or normal in NIDDM patients. Introduction of GHRP-6 permitted further investigation of the functional properties of the somatotrophs. **Aim**: Determination of GH response to GHRP-6, GHRH and GHRP-6+GHRH in NIDDM patients. **Materials and Methods**: Twenty-one NIDDM patient was studied, divided into two groups: Group A (Non-obese, n=8, age: 52.37±2.91 years, BMI: 23.31±0.62 kg/m^2) and Group B (Obese, n=13, age: 49.15±1.76, BMI: 27.62±0.72) and compared with healthy controls (Group C; n=8, age: 48.50±2.51, BMI: 23.78±0.70). GH response (fluoroimmunoassay, mIU/l, Delfia, Finland) was measured in three separate tests: GHRP-6 (90μg iv); GHRH (100μg iv) and GHRP-6+GHRH at -30;-15;0;15;30;45;60;90 and 120 min. **Results**: GH response was expressed as area under the curve (AUC). There was no difference after GHRP-6 in Group A, B and C concerning AUC for GH (2340.06±617.36; 2684.54±560.57; 3462.78±1223.53 mIU/l/120 min; $p>0.05$). GH response to GHRH was as follows in Group A, B and C: 1443.21±743.76; 479.62±84.00; 1476.51±386.56. There was difference among Groups B and A ($p<0.05$) as well as among Groups B and C ($p<0.05$) while there was no difference among Groups A and C ($p>0.05$). Administration of GHRP-6+GHRH elicited synergistic GH response both in NIDDM patients (Group A: 5111.13±703.77; Group B: 3425.95±459.67) and Group C: 9274.71±1541.46. There was no difference among Groups A and B ($p>0.05$) as well as among Groups A and C ($p>0.05$) while there was difference among Groups B and C ($p<0.05$). **Conclusions**: Non-obese NIDDM patients have preserved GH response to GHRP-6, GHRH and GHRP-6+GHRH while obese NIDDM patients have blunted response to GHRH and GHRP-6+GHRH. Preserved GH response to GHRP-6 in both diabetic subgroups suggests that the secretory potential of somatotrophs is preserved in NIDDM patients.

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DECONVOLUTION ANALYSIS OF GROWTH HORMONE (GH) RESPONSES TO GHRH AND GHRP-6 IN TYPE 1 DIABETES MELLITUS.

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In type 1 diabetes mellitus (DM 1), high GH basal levels and exaggerated GH responses to several stimuli, including GHRH and GHRP-6, have been described. Plasma GH level at any particular time is determined not only by its somatotroph secretion rate, but also by factors related to hormonal distribution and elimination phenomena.

Our aim was to elucidate if these high GH levels observed in DM 1 are really debt to an augmented pituitary secretion.

Six type 1 diabetic males (28.3±2.1 yr, 24.3±0.7 kg/m^2) and six age-, sex-, and body mass index-matched control volunteers (28.6±1.1 yr, 23.4±0.5 kg/m^2) were studied. Each subject received GHRH (100 μg iv), GHRP-6 (90 μg iv), and GHRH plus GHRP-6 on separate days. Serum GH levels were measured by IRMA at different times after every stimuli. A non-parametric deconvolution algorithm was used to analyse serum GH responses to GHRH and GHRP-6 and obtain an indirect *in vivo* estimation of the GH secretory pattern. GH half-lives employed in this study were previously assessed in DM 1 and normal subjects, using a bicompartmental analysis (5.9±0.8 and 34.5±7.5 min; 2.3±0.2 and 15.8±1.2 min, respectively).

We observed that the total amount of secreted GH after every stimulation was similar in both groups, as well as the time spent to secrete the hormone. After the combined administration of GHRH and GHRP-6 the number of deconvolution peaks was higher and the maximal peak of deconvolution showed an augmented amplitude and a reduced duration in DM 1 patients.

In conclusion, increased GH serum levels observed in DM 1 patients after the stimulation with GHRH and GHRP-6 might be mainly caused by alterations in its elimination kinetics, because there are not substancial differences on the GH secretory pattern respect to normal subjects.

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PLASMA GROWTH HORMONE LEVELS IN PATIENTS WITH NEWLY DIAGNOSED INSULIN-DEPENDENT DIABETES

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Aims: The onset of IDDM is frequently associated with higher then average height of patients. Therefore, the aim of this study was to investigate the plasma growth hormone (GH) levels in patients with newly diagnosed IDDM. ***Materials and Methods:*** We studied 79 patients with newly diagnosed IDDM aged from 3 to 15 years and 20 age-matched controls. GH measurement was performed at basal conditions and after administration of GH-releasing hormone (GH-RH) or 2 hours after beginning of sleep at night using specific radioimmune assay. The results between groups were compared using Student's paired test. ***Results:*** In 23.1% of children with diabetes the height was higher than average for respective age group. We found that basal plasma GH was significantly elevated in patients with newly diagnosed IDDM compared to control subjects - 8.58±0.78 and 2.05±0.46 ng/ml (data presented as mean±**SEM**), respectively, $p<0.001$. However, 30 minutes after administration of GH-RH levels of plasma GH did not differ between diabetic and control subjects - 21.8±0.34 and 20.5±0.46 ng/ml, respectively, $p>0.05$. The night GH levels were also higher in those with diabetes compared to healthy controls - 20.8±0.34 and 4.53±0.41 ng/ml, respectively, $p<0.01$. Consequently, we performed separate comparison of basal GH levels in adolescents 13-15 years old - GH levels were significantly elevated in diabetic patients compared to age-matched subjects - 9.83±0.37 and 3.78±0.37 ng/ml, respectively, $p<0.01$. ***Conclusions:*** Onset of IDDM is associated with increased production of GH either at basal condition or at night during the sleep while GH-RH induced GH levels did not differ in patients with newly diagnosed IDDM compared to age-matched controls. We speculate that enhanced secretion of GH may contribute to clinical manifestation of IDDM in children and adolescents.

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Thyroid, Fatty Acids and Lipolysis

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TRIIODOTHYRONINE: A LINK BETWEEN THE INSULIN RESISTANCE SYNDROME AND BLOOD PRESSURE?

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Overall obesity is associated with elevated serum triiodothyronine concentrations and insulin resistance. Oral triiodothyronine is known to induce hypertension in laboratory rats, while triiodothyronine also increases the expression of genes encoding for enzymes involved in the synthesis and secretion of insulin by pancreatic beta cells. **Aims:** To test the hypothesis that elevated free serum triiodothyronine concentrations link central obesity and insulin resistance with increased blood pressure and insulin production. **Materials and Methods:** We performed a cross-sectional study in 47 healthy euthryroid subjects (17 men, 30 women, mean ± SD age 34 ± 15 years).The waist-to-hip ratio was used as measure of central obesity, and insulin-stimulated glucose disposal during a hyperinsulinaemic euglycaemic clamp was used as measure of insulin sensitivity. Insulin production was calculated from the insulin clearance during the clamp and fasting insulin concentrations. **Results:** Free serum triiodothryronine concentrations correlated with systolic ($r = 0.40$, $P < 0.01$) and diastolic blood pressure ($r = 0.39$, $P < 0.05$), insulin production ($r = 0.41$, $P < 0.01$) and fasting insulin ($r = 0.54$, $P = 0.001$), independent of age and gender. There was only a borderline significant correlation of free serum triiodothyronine with the waist-to-hip ratio ($r = 0.30$, $P = 0.05$), and no correlation with insulin sensitivity as assessed during the clamp ($r = 0.23$, $P = 0.14$). The correlations of free serum triiodothyronine with blood pressure, insulin production and fasting insulin were independent of the waist-to-hip ratio and insulin sensitivity. **Conclusion:** Free serum triiodothyronine is apparently not an intermediate factor in the insulin resistance syndrome, but should be considered a new determinant of blood pressure, insulin production, and fasting insulin in healthy euthyroid subjects.

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INSULIN-THYROID HORMONE INTERACTION ON GLUCOSE PRODUCTION AND UTILIZATION IN HUMANS

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Aims:The effect of thyroid status on the glucose metabolism in humans was investigated in six hypothyroid patients before and after treatment, and six healthy volunteers before and after induction of hyperthyroidism. Materials and methods: All subjects were studied in the postabsorptive state and under hyperinsulinemic euglycemic and eukaliemic conditions. The insulin infusion rate was 6.5 ± 0.3 $pmol.kg^{-1}.min^{-1}$ for 140 min. An amino acid infusion was used to blunt insulin-induced hypoaminoacidemia. Continuous infusion of [6,6-$^2H_2$] glucose was used to measure glucose turnover. Results: In basal condition, glucose utilization (also an estimate of glucose production) increased from hypothyroid to euthyroid and further to hyperthyroid state (1.96 ± 0.15; 2.19 ± 0.18; 2.37 ± 0.07 $mg.kg^{-1}.min^{-1}$, respectively). Insulin infusion increased glucose utilization in all subjects. Assuming a dose response curve of glucose utilization as a function of plasma insulin, the initial slope of this curve yields an index of insulin sensitivity. Our results indicated that in the hypothyroid patients, the sensitivity of glucose utilization decreased but not significantly (1.91 ± 0.66 vs 4.78 ± 1.69 $min^{-1}.kg^{-1}$/ nmol $insulin.l^{-1}$) whereas this sensitivity is higher in the hyperthyroid state (8.02 ± 1.28 vs 5.26 ± 0.92 $min^{-1}.kg^{-1}$/ nmol $insulin.l^{-1}$, $P<0.05$). Accordingly, the sensitivity of glucose utilization to insulin linearly increased as a function of plasmaT3 level ($p=0.012$); the glucose production was completely inhibited during the infusion. The sensitivity of glucose production to insulin, was also a function of the T3 level ($P=0.048$).Conclusion: These results clearly demonstrated the crucial role of thyroid status in humans (from hypothyroidism to hyperthyroidism) on the glucose metabolism and the regulation by insulin of glucose production and utilization

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INSULIN SENSITIVITY AND FATTY ACID OXIDATION IN DIETARY INDUCED HYPERTRIGLYCERIDEMIA

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Aims: It has been proposed that increased storage of lipids in muscle is associated with insulin resistance (IR). To shed more light on the role of lipid metabolism in this situation, the fatty acid oxidation was assessed in relation to insulin sensitivity.

Material and methods: Total fatty acid (FA) oxidation (acid soluble products of palmitoyl-CoA) was measured in post-nuclear fractions from muscle and liver of rats fed either a basal chow diet (BD), or the insulin resistance (IR) -inducing high (70 cal%) fat (HF) diet for 3 weeks. In addition, a group of rats was fed the n-3 PUFA (Triomar, Norway) supplemented HF diet (HF+FO) in order to lower the Tg and FFA availability, and to improve insulin sensitivity. *In vivo* insulin action was assessed by an euglycemic hyperinsulinemic (6.4 mU/kg/min) clamp. **Results:** Feeding the animals HF diet led to an approx. 2-fold increase in postprandial plasma Tg [BD: 2.3±0.3 vs HF: 3.8±0.5 $mmol.l^{-1}$] and fasted plasma insulin [BD: 12.9±2.4 vs HF: 23.4±4.7 $mU.l^{-1}$], and to a 3-fold increase in fasted serum FFA [BD: 0.47±0.08 vs HF: 1.14±0.14 $mmol.l^{-1}$]. Supplementation of n-3 PUFA into the HF diet led to a significant decrease in postprandial Tg [HF+FO: 1.2±0.2], insulin [HF+FO: 13.1±3.2] and FFA [HF+FO: 0.65±0.17]. N-3 PUFA supplement into the HF diet was able to revert the decreased FA oxidation in liver [for palm-CoA BD: 0.38±0.05; HF: 0.08±0.01; HF+FO: 0.35±0.03 mmol/kg/min] in HF diet-induced IR to values seen in normal rats fed the BD. Simultaneously, a slight increase was found also in skeletal muscle [for Palm-CoA BD: 0.17±0.06; HF: 0.19±0.03; HF+FO: 0.25±0.02]. Insulin resistance, as evidenced by a 40 % decrease in glucose infusion rate (GIR) during the clamp, was present in the rats on the HF diet (BD: 26.3±2.2; HF: 15.7±0.4 mg/kg/min). In contrast, supplementation of the HF diet with n-3 PUFA improved the in vivo insulin action (HF+FO: 23.2±1.9). **Conclusions:** These data indicate that 1) the improvement of insulin action *in vivo* seen in rats with raised dietary intake of n-3 PUFA is associated with 2) a higher FA oxidation mainly in the liver which 3) may lead in turn to a lower Tg and FFA availability in circulation.

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EFFECTS OF MENTAL STRESS ON LIPOLYSIS IN ADIPOSE TISSUE AND IN SKELETAL MUSCLE

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Intramuscular lipids are increased in insulin resistant subjects. However, to date little is known about the in vivo metabolic regulation of these depots. Mental stress (MS) is known to augment lipolysis (LL) in adipose tissue (AT), as indicated by a higher interstitial glycerol (*glyc*) concentration. However, effects on muscular LL have not yet been explored. **Aim and methods:** To test whether and to what extent MS can augment LL in skeletal muscle (SM), *Glyc* was assessed before and following MS, induced by a color word test (CWT) for 10 min. Two double lumen microdialysis catheters (CMA 60) each were implanted in the paraumbilical subcutaneous AT and in the medial portion of the tibialis anterior muscle, to dialyse interstitial fluid (ISF). Twelve healthy volunteers (8m/4f) with a mean age of 27years, participated in the study. After two baseline measurements (-20 and 0min), ISF and serum samples were taken every 20 min for another hour, tissue blood flow (TF) was assessed with the ethanol wash-out technique. **Results:** Absolute ISF concentrations of *Glyc* were about six times higher in the AT vs SM; however, when increased *TF* in tibialis antior was taken into account, there was substantial release of *Glyc* in SM in the basal state. After 20-40 min of CWT, the *Glyc* rose significantly in AT(56%) and muscle(36%), while serum values remained unchanged. FFA increased by 40%. One hour after cessation of MS, these variables returned to normal in AT but were still elevated in SM. *TF* remained unchanged throughout the observation. **Conclusion:** MS stimulates LL in AT and also in SM. As muscle *TF* remained unchanged, the marked increase in *Glyc* cannot be attributed to decreased tissue perfusion, but rather indicates an augmented *Glyc* production. These data suggest that lipids in SM are subject to hormonal regulation. Therefore one might speculate that an increased lipid content in the SM could be involved in the development of insulin resistance.

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THE ANTILIPOLYTIC EFFECT OF INSULIN IN OBESE WOMEN BEFORE AND DURING SEMI-STARVATION

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Aims: Inhibition of lipolysis by insulin has been demonstrated in both adipose tissue and skeletal muscle. Semi-starvation of obese subjects is characterized by accelerated resistance to insulin regarding glucose metabolism. This study was undertaken to investigate the effect of caloric restriction on insulin induced-antilipolysis in obesity in vivo.

Material and Methods: Ten obese women (BMI 37.9±0.7 kg/m2, 61±3% fat) were investigated with microdialysis of abdominal adipose tissue and the gastrocnemius muscle. The absolute glycerol concentrations in arterialized plasma and the two tissue compartments were determined during a 2-step euglycaemic, hyperinsulinaemic (0.15 and 2 mU/kg/min) clamp. The clamp procedure was repeated after 7-10 days of VLCD (400 kcal). During caloric restriction the body weight was reduced by 3.9±0.6%.

Results: The basal glycerol concentrations were 301±28, 133±20 and 60±9 µmol/l in fat, muscle and plasma respectively (ANOVA p=0.0001 between compartments). This relationship was preserved during semi-starvation and the respective concentrations did not change significantly (280±24, 150±15 and 89±5 µmol/l). During the low insulin infusion rate glycerol levels decreased by 30%, both before and during semi-starvation, similarly in the three compartments. At the higher insulin infusion rate, before semi-starvation, there was a further decrease in glycerol levels (to 50% of basal; ANOVA repeated measurements p=0.0001). However, during caloric restriction there was no further reduction in glycerol levels at the high insulin infusion rate in either compartment.

Conclusions: In obese subjects the antilipolytic effect of insulin is similar in skeletal muscle and adipose tissue. During starvation the antilipolytic effect of insulin is reduced to a similar extent in fat and muscle. To further evaluate the impact of these findings on the whole body lipolysis rate measurements of the adipose tissue and skeletal muscle blood flow will be included.

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DIFFERENTIAL REGULATION OF SYSTEMIC, SUBCUTANEOUS ADIPOSE TISSUE AND INTRAMUSCULAR LIPOLYSIS IN MAN.

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The regulation of lipolysis is pivotal for many metabolic processes. In addition to visceral and subcutaneous adipose tissue muscle has been shown to contain measurable triglyceride stores. **Aims:** To assess the contribution of intramuscular and subcutaneous fat to the regulation of lipolysis during a 3-step hyperinsulinemic-euglycemic clamp (0.1, 0.25, 1.0 mU/kg/min). **Methods:** We used a combination of isotope (primed-continuous infusion of [d5]glycerol) and microdialysis techniques (catheters in the anterior tibial muscle and subcutaneous abdominal fat) in 13 lean healthy subjects. Glycerol rate of appearance (Ra) was used as index for systemic lipolysis and interstitial glycerol concentration as index for muscle (M) and subcutaneous fat (F) lipolysis. Insulin sensitivity in each compartment was expressed as the serum insulin achieving a half-maximal effect (EC50) and maximal decrease (max dec) during highest insulin infusion rate as % of basal. Metabolic clearance rate (MCR) of glucose was calculated at the highest insulin level.

Results: (p's indicate *Ra vs M, **Ra vs F, ***M vs F)

	Ra	M	F	p*	p**	p***
EC50(µU/ml)	7.0±1.0	5.8±0.9	8.8±1.2	0.001	0.001	0.001
max dec (%)	70±2	40±6	77±3	0.001	0.04	0.001

A nonlinear correlation between MCR and the EC50 Ra (r=0.93), M (r=0.85) and F (r=0.95) was found. **Conclusions**: Lipolysis in muscle is modulated by significantly lower insulin concentrations than in subcutaneous adipose tissue. It appears possible that regulation of intramuscular lipolysis contributes more to fine-tuning of systemic lipolysis particularly in the physiologic insulin range. Regulation of muscle lipolysis may have important consequences for muscle glucose metabolism.

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DECREASED LIPID OXIDATION AND FAILURE TO IMPROVE INSULIN SENSITIVITY PREDICT WEIGHT REGAIN

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Aims: To see if changes in body fat distribution, insulin sensitivity or substrate utilisation during weight loss are determinants of subsequent weight regain. **Methods:** 18 obese non-diabetic subjects underwent 12w of dietary intervention (DI), either a very low calorie diet (VLCD) or a low fat diet (LFD) regime. Body wt, subcutaneous abdominal (sc) and visceral (vis) fat areas by CT scan, glucose infusion rate (GIR) during a euglycemic, hyperinsulinemic clamp, % glucose (GOX) and % lipid oxidation (LOX) rates both before (basal) and during (IS) the clamp were assessed pre and post DI. Subjects were weighed 12m post DI. **Results:** Whereas those on VLCD lost more weight during DI (VLCD: -12.5±1.7, LFD: -6.3±1.1kg, p=0.0033), there was no difference between the mean body wt of the 2 groups 12m post DI (VLCD: 103.1±4.1, LFD: 106.8±3.7 kg. ns). Weight loss during DI was strongly correlated with improvements in GIR ($r = 0.526$, $p = 0.0250$) and loss of both vis ($r = 0.661$, $p = 0.0028$) and sc ($r = 0.744$, $p = 0.0004$) fat. Greater weight loss maintenance at 12m post DI was seen in those with the greatest increase in GIR during DI ($r = -0.717$, $p = 0.0008$) and in those who maintained higher basal LOX ($r = -0.495$, $p = 0.0366$) during DI. These subjects also had the highest basal LOX ($r = -0.666$, $p = 0.0025$) and lowest basal GOX ($r = 0.637$, $p = 0.0045$) post DI. Greater weight loss after 12m was not dependent on whether greater vis or sc loss had been achieved during DI. **Conclusions:** Lower lipid oxidation rates both during and after DI and a failure to improve insulin sensitivity during DI may be determinants of subsequent weight regain.

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THE IMPORTANCE OF HORMONE-SENSITIVE LIPASE AND FATTY ACIDS IN MODULATION OF INSULIN SECRETION

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Hyperglycemia together with elevated circulating free fatty acids (FFA) is believed to be involved in the pathogenesis of NIDDM. Chronically elevated fatty acids have adverse effect on insulin secretion and islets that overstore triglycerides are dysfunctional. On the other hand, islets that are depleted of their triglyceride stores have a blunted glucose-stimulated insulin secretion (GSIS), suggesting that a lipid-derived signal is involved in stimulus-secretion coupling in the β-cell. Data on how FFA are mobilized in β-cells and how this process is regulated is still scarce. In adipose tissue FFA are mobilized through the action of hormone-sensitive lipase (HSL), after its activation by cAMP-dependent protein kinase phosphorylation. Recently we found that HSL is expressed in β-cells and accounts for a significant part of the lipolytic activity in these cells. The aim of this study was to investigate the short- and longterm regulation of HSL in β-cells in relation to GSIS. **Methods**: The β-cell line INS-1 cells was cultured in serum-free RPMI 1640 medium (11.1. mM glc) in the presence of 0.5 mM fatty acids (palmitate/oleate 2:1) for 60 hours. The cells were then stimulated with forskolin and the degree of activation of HSL was monitored through measurement of triglyceride lipase activity. In other experiments, INS-1 cells were incubated with high (25 mM) respectively low (3.3 mM) concentrations of glucose for 4, 8, 16 and 32 h. The expression of HSL was monitored using quantitative Western blot analysis and total diglyceride lipase activity was estimated. **Results**: Triglyceride lipase activity was increased after stimulation of cells with forskolin. Preincubation with FFA resulted in increased GSIS and higher basal as well as stimulated triglyceride lipase activity. High glucose was found to induce the expression of HSL in INS-1 cells two-fold whereas the total diglyceride lipase activity was elevated 1.5-fold under the same conditions. **Conclusions**: Activation of HSL in response to forskolin-stimulation of INS-1 cells suggests that the enzyme, via hydrolysis of intracellular triglyceride stores, generates a lipid-derived signal that contributes to the release of insulin. Furthermore, the induction of HSL expression in response to high glucose concentrations, may reflect an adaptation of the cell to the hyperglycemic state.

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THE ROLE OF DIABETES IN DELAYED CHYLOMICRON CLEARANCE

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Aim To disassociate the diabetic state from the alteration in chylomicron composition caused by diabetes in an examination of chylomicron clearance. **Materials and Methods** 5 Cholesterol fed alloxan diabetic rabbits and 5 control rabbits were given by gavage [^{3}H] cholesterol and [^{14}C] linoleic acid to produce dual labelled chylomicrons for turnover. The lymph duct was cannulated and radiolabelled chylomicrons collected. Lipids and Apo B48 and apo B100 were determined. Chylomicrons from control rabbits were injected into paired control (cc) and diabetic (cd) rabbits and chylomicrons from diabetic rabbits were injected into paired control (dc) and diabetic rabbits (dd). Plasma was collected at intervals for up to 40 min and radioactivity counted. **Results** Chylomicron apo B48 and B100 were higher in the diabetic animals (38.5±1.9 vs 4.88±0.5 µg/ml lymph and 46.1±4.3 vs 7.3±1.4 µg/ml lymph p<0.0001) but there was no difference in chylomicron lipids. The early phase of chylomicron cholesterol clearance was fastest in cc and slowest in dd animals (p<0.003). dc and cd were intermediate (ns). At 40 minutes clearance was greatest in cc and least in both dd and cd, dc being intermediate. Similar results were obtained on examination of the triglyceride label. **Conclusion** Delayed chylomicron clearance in diabetes is associated with an increase in chylomicron apo B48 and B100 but also contains a compositional independent factor since chylomicrons from control rabbits were cleared more slowly when injected into diabetic animals.

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EXAGGERATED DIURNAL TRIGLYCERIDE PROFILES AS EARLY SIGN OF DIMINISHED INSULIN SENSITIVITY IN NORMOLIPIDEMIC MALES.

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Aims: Since decreased insulin sensitivity is associated to fasting and postprandial hyperlipidemia we hypothesized that subtle changes in diurnal triglyceride (TG) metabolism might be related to insulin sensitivity. In addition, the effect of age, body composition and diet was assessed in relation to diurnal TG profiles.

Materials and Methods: Forty-eight normolipidemic, non-obese, healthy males (20-55 yrs) measured the diurnal capillary TG (TGc), for three days, six times a day using the Accutrend GCT and recorded their food intake. Fasting blood was collected once. Body composition was measured by body impedance. Diurnal TGc-profiles were calculated as area under the mean TGc curve of three days (TG-AUC).

Results: The mean TG-AUC was 23.6±6.7 mmol.hrs/l. Age did not influence diurnal TGc profiles, since there was no significant difference in TG-AUC between the youngest (age:20-35 yrs; n=30) and the oldest group (age:35-55 yrs; n=18): 24.6±6.7 and 22.0±6.5 mmol.hrs/L, respectively. The best determinants of TG-AUC were fasting TGc (r=0.74, P<0.005), systolic BP (r=0.40,P<0.005), fasting insulin (r=0.40, P<0.005), HOMA ratio (r=0.32,P<0.05), relative body fat (r=0.31,P<0.05) and total saturated fat intake (r=0.30,P<0.05). The best predictors of fasting TGc by multiple regression were systolic BP (β:0.37;P<0.05) and saturated fat intake as percentage of total energy intake (β:0.31;P<0.05). A subgroup (n=10) with elevated TG-AUC was identified. Compared to the rest of the group, the subgroup had a higher TG-AUC (33.5±2.2 vs. 21.1±4.8 mmol.hrs/l; p<0.005), fasting TGc (1.45±0.28 vs. 1.12±0.35 mM; p<0.005), fasting insulin (10.3±3.3 vs. 7.3±2.4 mU/l; p<0.005), HOMA-ratio (2.2±0.8 vs. 1.5±0.7; p<0.05) and protein intake (120±35 vs. 99±24 g/d; p<0.05).

Conclusions: Diurnal TGc-profiles in healthy normolipidemic males are not age-dependent, but depend on insulin sensitivity, body composition and diet. A disturbed diurnal TGc-profile could be the first sign of a developing metabolic syndrome.

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THE RELATIONSHIP BETWEEN INSULIN SENSITIVITY AND POSTPRANDIAL ADIPOSE TISSUE LIPID METABOLISM

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AIMS: It is suggested that decreased postprandial non-esterified fatty acid (NEFA) suppression is a component of the insulin resistance syndrome. We investigated insulin sensitivity (IS), postprandial NEFA release and triglyceride (TG) extraction at adipose tissue level. **Materials and Methods:** 32 subjects were given a meal (60 g fat, 85 g carbohydrate and 13 g protein). Blood was taken from a vein draining the anterior abdominal subcutaneous adipose tissue depot and an arterialised hand vein at -20, 0, 30, 60, 90, 120, 180, 240, 300 and 360 min. Arterialised plasma insulin and NEFA, glucose, TG concentrations in both samples were measured. BMI was calculated. Mean fasting arterialised glucose : insulin ratio was used as a measure of IS. % maximal decrease in NEFA release and % maximal increase in TG extraction across adipose tissue were calculated. **Results:** As expected, IS was correlated with BMI (r_s -0.61, $P < 0.001$). % maximal decrease in adipose tissue NEFA release was correlated with both BMI (r_s -0.44, $P = 0.013$) and IS (r_s 0.51, $P = 0.003$). There was a strong negative correlation between % maximal increase in TG extraction and % maximal decrease in NEFA release across adipose tissue (r_s -0.67, $P < 0.001$). Forward stepwise regression using BMI, IS and % maximal increase in TG extraction showed that the latter was responsible for 52% of % maximal NEFA suppression variability. **Conclusions:** This confirms that failure of suppression of adipose tissue postprandial NEFA release is a feature of obesity and of the insulin resistance syndrome. Perhaps unexpectedly, the more insulin sensitive subjects did not clear TG more efficiently after the meal and the ability to clear TG was predictive of a failure to suppress NEFA release after a meal. The resultant high postprandial NEFA concentrations in more obese, insulin resistant subjects may explain the increased risk of atherosclerosis in these individuals.

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VERY LOW DENSITY LIPOPROTEIN OVERSECRETION INCREASES WITH DEVELOPMENT OF GLUCOSE INTOLERANCE

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Aims: To further understand the kinetics of *in vivo* apolipoprotein (apo) B-100 turnover under insulin resistant conditions and during development of glucose intolerance. Materials and Methods: Two stable isotope studies (intra-individual comparison, 3-year follow up) were performed in 3 non-obese first degree relatives with initially normal glucose tolerance but a familial diagnosis of type 2 diabetes (F-group) and in 6 non-related controls. During and after a 12*hr* primed, constant infusion of either [$^{13}C_6$]-phenylalanine or [$^2H_3$]-leucine tracer enrichment was determined in apoB-100 from ultracentrifugally isolated $VLDL_1$ (S_f 60-400) and $VLDL_2$ (S_f 20-60). The rates of production, catabolism, and transfer were estimated by model-based multicompartmental analysis. Results: The first study showed hepatic $VLDL_1$ oversecretion completely responsible for higher triglyceride (TG) levels in the F-group (TG: 1.69 ± 0.81 *vs* 0.95 ± 0.21 mmol/L, $p<.05$; $VLDL_1$ apoB-100 production rate (PR): 739 ± 13 *vs* 617 ± 31 mg/d, $p<.01$). Three years later two of the 3 relatives had developed an impaired glucose tolerance (IGT), one an impaired fasting glucose (IFG). In the F-group, TG levels were higher by 7.1% ($p<.05$) and $VLDL_1$ apoB-100 PR were higher by 12.3% ($p<.05$), whereas in the controls no significant changes could be measured. The fractional catabolic rates of apoB-100 in $VLDL_1$ and $VLDL_2$ and the direct (hepatic) PR of $VLDL_2$ apoB-100 showed no differences and no changes in both groups, resp. In the F-group, the increment in $VLDL_1$ secretion is associated with an increment of plasma insulin (by 22.6%, $p<.01$) and free fatty acid (by 65.2%; $p<.01$) levels, resp., indicating a progressive loss of insulin sensitivity in this group. Conclusions: $VLDL_1$ oversecretion is an early indicator of an insulin resistant state, deteriorating with development of glucose intolerance.

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TRIGLYCERIDE KINETICS IN DARGLITAZONE-TREATED OBESE ZUCKER RATS.

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Thiazolidinediones, used in the treatment of type 2 diabetes, are effective anti-hyperlipidaemic agents in various animal models of insulin resistance. Aims: The present study was undertaken to elucidate the kinetic mechanisms responsible for the anti-hypertriglyceridaemic effect of a potent thiazolidinedione, darglitazone. Materials and Methods: Male obese Zucker rats were gavaged with darglitazone (1.3 µmole/kg/day) or vehicle for 3 weeks. At the end of the treatment period, Triton WR1339, which blocks the clearance of plasma triglycerides (TG), was administered to Inactin®-anaesthetised, 5 hr fasted animals and blood samples were collected for a 2 hr period. Results: Basal plasma TG was 8.9±1.3 mM in the vehicle control group and 0.9±0.17 mM in darglitazone-treated obese Zucker rats demonstrating a marked reduction in plasma TG (90%, P<0.05) with darglitazone treatment. Hepatic triglyceride output (HTGO), calculated from the slope of the linear rate of accumulation of TG in plasma after the administration of Triton, was decreased by 42% in the darglitazone-treated animals (HTGO:1.8±0.04 vs. 3.1±0.12 µmol/min in the vehicle group, P<0.05). In addition, plasma TG clearance rate (K), an index of the effectiveness of tissues to remove TG from the circulation, (estimated as the ratio of HTGO to basal plasma TG concentration sampled immediately before Triton administration) was ~9-fold increased in the darglitazone-treated animals (K:3.5±0.44 vs. 0.4±0.06ml/min in the vehicle group, P<0.05). Size distribution of lipoproteins obtained by gel-permeation chromatography revealed that the control animals were characterised by a large "VLDL"-peak, and relatively low "IDL/LDL"- and high "HDL"-peaks. Darglitazone virtually abolished the "VLDL"-peak, increased the "IDL/LDL" without changing the "HDL" fraction. Following Triton, selective changes were seen in the "VLDL"-peak, which was restored in the darglitazone-treated animals and increased in amplitude in the control animals. Conclusions: The present study demonstrates that darglitazone-induced lowering of hypertriglyceridaemia in obese Zucker rats involves both a decrease in hepatic triglyceride output as well as an increased triglyceride clearance. This increased clearance involves accelerated conversion of newly secreted VLDL particles to smaller lipoprotein species.

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A NOVEL LOSS OF FUNCTION MUTATION OF LIPOPROTEIN LIPASE [Cys239→Trp] ASSOCIATED WITH TYPE I HYPERLIPOPROTEINEMIA (HLP) IN A PATIENT WITH RECURRENT SEVERE PANCREATITIS.

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Aims: Lipoprotein lipase (LPL) is the major enzyme responsible for the hydrolysis of triglyceride (TG)-rich lipoproteins in plasma. The purpose of this study was to examine the molecular pathogenesis of type I HLP (TG: 1200-4000 mg/dl) in a patient suffering from recurrent severe pancreatitis.

Methods and Results: ApoCII-concentration was normal and apoCII did activate LPL in an *in vitro* assay. In post heparin plasma neither LPL mass nor activity was detectable, whereas hepatic lipase activity was normal. Direct sequencing of all ten exons of the LPL gene revealed that the patient was homozygous for a hitherto unknown mutation in exon 6, Cys239→Trp. The mutation prevents the formation of the second disulfide bridge of LPL, which is an essential part of the lid covering the catalytic center. Consequently, misfolded LPL is rapidly degraded within the cells, causing the absence of LPL-immunoreactive protein in the plasma of this patient. Euglycemic hyperinsulinemic glucose clamp studies revealed normal insulin sensitivity with a MCR of glucose of 8.4 ml/kg/min, although fasting and steady state free fatty acids (FFA) were markedly elevated. Furthermore, insulin-induced antilipolysis was not impaired.

Conclusion: We have identified a novel loss of function mutation in the LPL-gene (Cys239→Trp) of a patient with type I HLP suffering from severe recurrent pancreatitis. Despite excessive TG+FFA elevations, insulin sensitivity was not impaired.

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RELATIONSHIP BETWEEN PLASMA FREE FATTY ACIDS AND UNCOUPLING PROTEIN-3 GENE EXPRESSION IN SKELETAL MUSCLE OF OBESE SUBJECTS.

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Uncoupling protein-3 (UCP3) is a recently discovered mitochondrial protein expressed in skeletal muscle, a major site of thermogenesis in adult humans. By uncoupling respiration from ATP synthesis UCP3 may be involved in the control of energy expenditure. Furthermore, UCP3 mRNA levels are upregulated during fasting, a finding that has been linked to changes in plasma free fatty acids (FFA). **Aims**: to investigate whether skeletal muscle UCP3 gene expression: 1) is altered in obesity; 2) correlates with in vivo insulin sensitivity; 3) correlates with plasma FFA. **Materials and Methods**: we measured UCP3 mRNA levels in skeletal muscle of 12 obese, non diabetic, non hypertensive subjects (10F/2M; aged 32.3±3.7 yr.; range 19-53; BMI 42.4±1.2 kg/m^2; mean±SEM) and 8 healthy lean control subjects (6F/2M; aged 38.6±4.1 yr.; range 25-64; BMI 23.6±1.3 kg/m^2; mean±SEM) undergoing euglycemic clamp. Skeletal muscle UCP3 mRNA levels were measured by reverse transcriptase-competitive polymerase chain reaction. **Results**: no significant differences in UCP3 mRNA levels were found between obese and control subjects (0.25±0.09 vs 0.24±0.08 amol/µg RNA respectively). Furthermore, no significant correlations were observed, in both groups, between UCP3 mRNA levels and BMI (r= -0.58, p= 0.1), percent fat mass (r= 0.36, p= 0.38), waist to hip ratio (r= -0.02, p= 0.96), glucose disposal rate (r= 0.17, p= 0.67), plasma leptin (r= 0.29, p= 0.54) and fasting plasma insulin concentrations (r= -0.39, p=0.38). In contrast, a positive and significant correlation was observed between skeletal muscle UCP3 mRNA levels and plasma FFA (r= 0.83, p<0.04). **Conclusions**: 1) there is no major alteration of UCP3 gene expression in skeletal muscle of obese subjects; 2) UCP3 does not seem to influence insulin sensitivity; 3) FFA may regulate UCP3 gene expression.

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EFFECTS OF REXINOIDS AND THIAZOLIDINEDIONE ON LEVELS OF UCP ISOFORMS AND PGC-1 IN THE OBESE ZUCKER RAT

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The mitochondrial uncoupling protein UCP-1 is essential for the thermogenic function of brown adipose tissue (BAT), due to its ability to uncouple oxidative phosphorylation from ATP synthesis. This results in energy being dissipated as heat. Two further isoforms, UCP-2 and UCP-3, have been identified in other tissues. The retinoid X receptor (RXR) for 9-cis-retinoic acid forms heterodimers with several nuclear receptors, including the peroxisome proliferator-activated receptor gamma (PPARγ). We have demonstrated that RXR ligands (rexinoids) improve insulin sensitivity and reduce body weight. PPARγ is the functional receptor for thiazolidinediones which also improve insulin sensitivity. However in contrast to the rexinoids, administration of the thiazolidinedione results in a moderate weight gain. **Aims**: To investigate the effects of rexinoids and thiazolidinedione on levels of UCP isoforms and the recently described PPARγ co-activator (PGC-1) and correlate changes with the effects of these drugs on whole body physiology. **Material and Methods**: Zucker *fa/fa* rats were dosed orally for two weeks with the rexinoids LG100268 or LG100324 (20mg/kg) or the thiazolidinedione BRL49653 (3mg/kg). mRNA levels were assessed by quantitative PCR. **Results**: A partial cDNA sequence of the rat PGC-1 coding sequence was identified. Treatment with the rexinoids or the thiazolidinedione had no effect on PGC-1 or UCP-2 mRNA levels in BAT, skeletal muscle, white adipose tissue or brain. BAT UCP-1 was increased 2.7 fold (p<0.002) by LG100268 and 3.1 fold (p<0.001) by LG100324 whilst treatment with BRL49653 was without effect. Following treatment with LG100324 UCP-3 mRNA was increased by 3.6 fold (p<0.0005) in muscle and 4.3 fold (p<0.0002) in WAT. LG100268 increased UCP-3 in WAT by 2 fold (p<0.005) but had insignificant effect on muscle UCP-3. BRL49653 increased expression of UCP-3 by 2.1 fold (p<0.005) in muscle and by 2.7 fold (p<0.003) in WAT. **Conclusions**: The rexinoids affect UCP expression more potently than the thiazolidinedione. This is particularly apparent in their effect on BAT UCP-1 that could in part account for the weight reducing effects of the rexinoids. Our result also suggest that the PPARγ co-activator PGC-1 gene is not a target of neither the rexinoids nor the thiazolidinedione.

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FATTY ACID COMPOSITION AND SUSCEPTIBILITY OF LDL TO OXIDATIVE MODIFICATION - EFFECTS OF ACUTE HYPERGLYCAEMIA AND HYPERTRIGLYCERIDAEMIA.

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Increased oxidative modification of low-density lipoproteins (LDL) has been implicated as independent risk factor for atherosclerosis. LDL oxidation in vitro is stimulated by glucose itself and is influenced by dietary polyunsaturates. **Aims:** The aim of our study was to evaluate the effect of acutely-induced hyperglycaemia and hypertriglyceridaemia on LDL oxidizability, and to explore the role of the fatty acid (FA) pattern in healthy subjects. **Materials and Methods**: The susceptibility of LDL to Cu^{2+} induced oxidative modification and FA composition of LDL phosphatidylcholine (PL) and cholesterol esters (CHE) were measured in 13 healthy men under following conditions lasting 4 hours: a) hyperglycaemic clamp (12 mmol/l; HG), b) hyperglycaemic clamp with infusion of somatostatin in order to decrease the insulin production (HGS), c) infusion of Intralipid (0.15 g of fat.kg^{-1}.h^{-1}; I), and d) a time-controlled study with saline infusion (C). **Results:** Conjugated diene production did not change during HG and HGS compared to C (HG: 564 ± 75 vs 560 ± 76 nmol/mg of LDL protein; HGS: 564 ± 70 vs 582 ± 72 mmol/mg of LDL protein; C: 443 ± 85 vs 450 ± 70 mmol/ mg of LDL protein), while it significantly decreased during I (589 ± 100 vs 524 ± 87 mmol/ mg of LDL protein; $p < 0.05$). No significant changes in the lag time of diene production (HG: 74.81 ± 27.94 vs 76.19 ± 26.73 min; HGS: 66.9 ± 9.35 vs 69.21 ± 15 min; I: 69.03 ± 22.3 vs 86.82 ± 18.30 min; C: 68.58 ± 14.57 vs 74.81 ± 19.79 min), in thiobarbituric acid reactive substances concentration and in emission fluorescence spectra intensity were found. The FA pattern of PL and CHE remained unchanged during studies and was not associated with LDL oxidizability in basal state. **Conclusions:** The results suggest that 4 hour in vivo induced hyperglycaemia or hypertriglyceridaemia do not change the FA composition of LDL and are not able to increase the LDL oxidizability. Despite high content of polyunsaturates in lipid emulsion, infusion of Intralipid has even the tendency to decrease LDL oxidizability probably due to high tocopherol content in lipid emulsion. (Supported by grants IGA MZ ČR No. 4903-3 and 4888-3).

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THE ROLE OF BODY FAT DISTRIBUTION IN THE ACUTE EFFECTS OF ORAL GLUCOSE STIMULATED INSULIN ON LIPID METABOLISM

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Aims: To find out the acute effects of oral glucose stimulated insulin on lipid metabolism of normal weight and obese individuals in relation with body fat distribution. **Materials and Methods:** 67 individuals (49 women and 18 men) in three age-matched groups were participated in the study. 20 (14 women, 6 men) abdominal obese cases with BMI>27 and waist to-hip ratio (WHR)>0.95 (in men), >0.85(in women); 21 (17 women, 4 men) general obese cases with BMI>27 and WHR<0.95 (in men),<0.85 (in women); 26 (18 women, 8men) non obese cases with BMI<27 and WHR<0.95 (in men) and<0.85 (in women) were made up the groups. OGTT with 75g of glucose was performed.Plasma glucose at 0,1,2,3,4 h; plasma insulin at 0,1,2,h; total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol at 0,4 h.were measured during OGTT **Results:**Fasting insulin, insulin area under OGTT,0.,1.,2. h insulinogenic indices in the abdominal obese group were significantly ($p<0.001$) higher than in the non obese group. These parameters were also higher in the general obese group than in the non obese but significance were lower. There were also significant difference between the abdominal obese and the general obese group in fasting insulin and 0.h insulinogenic index ($p<0.001$ and $p<0.01$ respectively). It was observed that total cholesterol, triglyceride, LDL-cholesterol were decreased (%2.28, %10.65, %3.68 respectively) and HDL-cholesterol increased (%21.47) at the 4^{th} h of OGTT in accordance with baseline. On the other hand there were increases in total cholesterol, triglyceride, LDL-cholesterol and decrease in HDL-cholesterol at the 4^{th} h of OGTT in both obese groups but significance was higher in the abdominal obese group ($p<0.01$ vs $p<0.05$). **Conclusion:** Acute hyperinsulinemia has suppressive effects on lipids and lipoproteins except HDL in normal weight cases. These suppressive effects have not been observed in both obese groups particularly in the abdominal obese group showing the resistance to insulin effect.

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INSULIN SENSITIVITY IS AN INDEPENDENT DETERMINANT OF LDL SIZE IN HEALTHY POPULATION (THE KANWU STUDY)

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Aims: To define the independent influence of insulin sensitivity and plasma triglyceride (TG) levels on LDL size in a large sample of healthy individuals representative of population groups with different genetic background and/or life style (Kuopio, Aarhus, Naples, Wollongong, Uppsala).

Materials: 162 non diabetic, non hyperlipidemic subjects (86 men, 76 women, age 49 ± 8, BMI 26 ± 3 kg/m^2) (M±SD).

Methods: Insulin sensitivity index (SSI) was measured by the frequently sampled i.v. glucose tolerance (the minimal model according to Bergman). Furthemore fasting lipids, apoB, $apoA_1$, LDL size (gradient gel electrophoresis), post-heparin plasma lipoprotein lipase (LPL) and hepatic lipase (HL) activities (Nilsson-Ehle method) were determined.

Results: LDL size was significantly correlated with SSI ($r=0.30$, $p<0.0001$) and HDL cholesterol ($r=0.19$, $p<0.01$), while the correlation with TG was only of borderline significance ($r=-0.15$, $p=0.06$). No correlations were found between SSI and BMI ($r=-0.04$), LPL ($r=0.12$) and HL ($r=-0.008$). By multivariate analysis SSI remained independently related to LDL size ($p<0.001$) together with apoB concentration ($p<0.02$), while HDL cholesterol, TG and fasting insulin did not enter in the model (stepwise model).

Conclusions: Insulin sensitivity plays a major role in determining LDL size while TG, HDL cholesterol and insulin levels are of less importance. In a normal population factors influencing LDL size might be different from those operative in diabetic and hypertriglyceridemic individuals.

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PLASMA PHOSPHOLIPID TRANSFER PROTEIN ACTIVITY IS LOWERED BY 24 H INSULIN AND ACIPIMOX ADMINISTRATION: BLUNTED RESPONSE TO INSULIN IN TYPE 2 DIABETIC PATIENTS.

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Cholesteryl ester transfer protein (CETP) transfers cholesteryl ester from HDL to VLDL+LDL.Phospholipid transfer protein (PLTP) transfers phospholipids between lipoproteins, converts HDL_3 into larger and smaller particles, and is involved in preβ-HDL generation. The effects of 24 h hyperinsulinaemia (30 mU/kg/h) and 24 h Acipimox (250 mg per 4 h) on plasma lipids as well as on CETP and PLTP activities (measured with exogenous substrate assays) was examined in 8 healthy and 8 Type 2 diabetic subjects. After 24 h of insulin plasma free fatty acids (FFA), HDL cholesterol and plasma apolipoprotein AI decreased in both type 2 diabetic patients and healthy subjects ($p<0.05$). After 24 h of Acipimox all these parameters, including plasma triglycerides, decreased in both groups ($p<0.05$). Insulin decreased plasma PLTP activity by 17.6±6.0% at 24 h in healthy subjects ($p<0.05$) and 10.2±6.6% in diabetic patients ($p<0.05$ from baseline, $p<0.05$ from healthy subjects). Acipimox lowered PLTP activity by 10.3±9.4% ($p<0.05$) in healthy subjects and by 11.3±5.8% in diabetic patients ($p<0.05$). When insulin was infused for 3 h after Acipimox, a further decrease was found only in healthy subjects. Plasma CETP activity decreased by 9.5±6.4% after 24 h of insulin only in healthy subjects ($p<0.05$), but not in diabetic patients. Acipimox did not decrease plasma CETP activity in either group. The PLTP responses with insulin and Acipimox were larger than the changes in CETP activity in healthy subjects ($p<0.05$). In conclusion, there is a metabolic link between the regulation of plasma PLTP, but not CETP, and FFA. The PLTP response to insulin is blunted in Type 2 diabetes mellitus. The effects on PLTP activity may have consequences for HDL metabolism and reverse cholesterol transport.

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EFFICACY AND SAFETY OF CERIVASTATIN/FENOFIBRATE COMBINATION THERAPY FOR HYPERCHOLESTEROLEMIA

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Aims: To investigate the potential of cerivastatin/fenofibrate combination therapy for improving blood lipid levels in patients with primary hypercholesterolemia. **Materials and Methods:** This was a randomized, multinational, double-blind study. On entry, all patients had to have an LDL-cholesterol level ≥4.12 mmol/l (or ≥3.35 mmol/l if they had a history of CHD or 2 or more cardiovascular risk factors) and a fasting triglyceride level ≤3.99 mmol/l. All patients initially received placebo for 6 weeks, then they were randomized to 1 of 3 groups. Group 1 (CER, n = 115) received cerivastatin 0.3 mg/day alone for 16 weeks. Group 2 (FEN, n = 112) received fenofibrate micronized 200 mg/day alone for 16 weeks. Group 3 (CER + FEN, n = 115) received cerivastatin 0.3 mg/day alone for 8 weeks, then cerivastatin 0.3 mg/day and fenofibrate 200 mg/day for a further 8 weeks. **Results:** Percentage changes in efficacy variables in the ITT population (baseline to week 16):

Parameter	CER	FEN	CER + FEN
LDL-cholesterol	−27.6[1,2]	−21.0[2]	−40.5
Total cholesterol	−19.6[2,3]	−16.4[2]	−29.9
HDL-cholesterol	+5.8[1,2]	+12.0	+12.4
Triglycerides	−9.6[1,2]	−31.6	−37.2

[1]Significantly different from FEN (P < 0.01); [2]Significantly different from CER + FEN (P < 0.001); [3]Significantly different from FEN (P = 0.03).

The safety profile of cerivastatin/fenofibrate combination therapy showed no major differences from that of either drug used alone, apart from a moderate increase in the rate of transaminase elevations in the combined therapy group. **Conclusions:** Combined cerivastatin/fenofibrate therapy has greater efficacy than either drug used alone for improving the blood lipid profile of patients with dyslipidemia. The combination may be useful for decreasing LDL-cholesterol and triglyceride levels and increasing HDL-cholesterol levels in patients with type 2 diabetes.

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INFLUENCE OF INSULIN SENSITIVITY AND THE TAQIB CHOLESTERYL ESTER TRANSFER PROTEIN GENE POLYMORPHISM ON PLASMA LECITHIN: CHOLESTEROL ACYLTRANSFERASE AND LIPID TRANSFER PROTEIN ACTIVITIES AND THEIR RESPONSE TO HYPERINSULINAEMIA IN NON-DIABETIC MEN.

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HDL metabolism is governed by several factors, including lecithin cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), phospholipid transfer protein (PLTP) and lipases. We evaluated the influence of insulin sensitivity insulin infused at 30 mU/kg^{-1}h^{-1} during 3h and of the TaqIB CETP gene poly-morphism (an intronic polymorphism, B1B2) on plasma LCAT, CETP and PLTP activity levels and the responses of these factors to hyperinsulinaemia in 32 non-diabetic men. Plasma total cholesterol, VLDL + LDL cholesterol, triglycerides, and apo B levels were higher in the quartile of subjects with the lowest insulin sensitivity (Q_4) than in the subjects with the highest insulin sensitivity (Q_1) ($p<0.05$ for all), whereas HDL cholesterol and apo A1 was lowest in Q_4 ($p<0.05$ for both). Plasma LCAT activity was higher in Q_4 than in Q_1 ($p<0.05$) and PLTP activity was higher in Q_4 than in Q_2 ($p<0.05$), but insulin sensitivity did no influence plasma CETP activity. Plasma lipoprotein lipase activity was higher and hepatic lipase activity was lowest in Q_1. Insulin infusion did not affect plasma LCAT and CETP activity, but decreased PLTP activity similarly in the 4 quartiles of insulin sensitivity ($p<0.05$ for all). There were no differences in baseline plasma LCAT, CETP or PLTP activities between 11 B1B1 and 8 B2B2 homozygotes. After insulin plasma CETP activity had decreased slightly in B1B1 homozygotes only ($p<0.05$). The decrease in plasma PLTP activity was also larger in B1B1 than in B2B2 homozygotes ($p<0.05$). In conclusion, insulin sensitivity influences plasma LCAT, PLTP, lipoprotein lipase and hepatic lipase, but not CETP activities in men. High levels of hepatic lipase, PLTP and LCAT in association with insulin resistance may promote reverse cholesterol transport. The most pronounced decrease in plasma PLTP activity during physiological hyper-insulinaemia in B1B1 homozygotes could be associated with an altered reverse cholesterol transport.

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ABDOMINAL ADIPOSITY IN OVERWEIGHT INSULIN-DEPENDENT DIABETIC ADOLESCENTS

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Overweight in IDDM is not a rare event, especially in girls during adolescence. Insulin is involved in fat deposition but the physiological plasma insulin profile is lost in IDDM patients. ***Aim***: to evaluate body mass composition by MRI and DXA in obese female with or without IDDM. ***Materials and methods***: we have studied 13 IDDM obese female (IDDM-OB) and 11 obese female (OB). IDDM-OB and OB were similar regarding age 16.4 ± 2.0 vs 20.1± 3.9 yrs and BMI 27.2 ± 2.6 vs 27.4±4.5. Body mass composition was assessed by DXA and by MRI taken by lumbar (L4) level. Statistical analysis on subcutaneuos adipose tissue (SAT cm^2) and intra-abdominal adipose tissue (IAT cm^2) have been performed by non parametric methods. ***Results:*** SAT was comparable between IDDM-OB and OB: 260.7 (109.1-431.9) vs 305.7 (221.4 -560.0) cm^2 (p = n.s.); IAT was significantly lower in IDDM-OB than OB: 19.7 (10.9-35.7) vs 32.5 (11.4-51.1) cm^2 ($p < 0.01$). SAT was strongly related to IAT tissue in OB ($r = 0.7$; $p < 0.03$), but not in IDDM-OB. Fat, lean tissue and total bone mineral content assessed by DXA were similar between IDDM-OB and OB. Fat mass assessed by DXA was strongly related to SAT by MRI in OB ($r = 0.9$; $p < 0.03$) and in IDDM-OB ($r = 0.8$ $p < 0.01$) but not to IAT by MRI in both groups. ***Conclusions***: obesity in IDDM female is associated to lower intra-abdominal fat deposition than in weight-matched non diabetic female.

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ULTRASOUND IS A RELIABLE METHOD TO ASSESS THE AMOUNT OF ABDOMINAL FAT.

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Aims In recent years it has become clear that the amount of fat stored in the abdomen is associated with insulin resistance and increased risk of morbidity and mortality. Currently, the best assessment of abdominal fat is computed tomography (CT). The most often used measurements are waist circumference and the ratio of waist and hip circumferences (WHR). However, these do not clearly distinguish between subcutaneous and visceral fat. Ultrasound might be a suitable technique to obtain a more accurate method to assess the amount of abdominal fat without CT. We validated a new abdominal ultrasound protocol using CT as gold standard.

Materials and Methods The distance between peritoneum and lumbar spine is measured at five standard positions by a 10MHz transducer. All measurements are performed from one line over the abdomen halfway between the lower rib and iliac crest. Abdominal fat is assessed in a single CT-slice at L4-L5, by counting the tissue area with Hounsfield units between -150 and -50 inside the peritoneum.

Results The study population consisted of 21 overweight subjects (14 women, 7 men), mean age 42.9 years (SD 17.2), body mass index 33.0 kg/m^2 (4.1), waist/hip ratio 0.93 (0.009), and abdominal fat on CT 135.5 cm^2 (55.7). The mean anterior distance was 10.3 cm (2.0). There was a strong association between the CT and ultrasonographic measures. Pearson correlation coefficient of the mean anterior distances was 0.82 ($p<0.001$). The correlation between WHR and ultrasound was 0.71 ($p<0.001$), the correlation between CT and WHR was 0.57 ($p=0.01$). The correlation coefficient of the anterior measurements performed by two different sonographers was 0.90 ($p<0.001$), the mean difference 0.8 cm (1.2), indicating high reproducibility of the ultrasound measurements.

Conclusion The distances obtained by ultrasound were stronger associated with the amount of fat on CT than waist/hip ratio (and waist circumference). Because this non-invasive technique is widely available and the measurements take only a few minutes, it is a promising technique especially for clinical and epidemiological studies.

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Clinical Studies: Type 1 Diabetes

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CLINICAL PRESENTATION AND INITIAL EVOLUTION OF IDIOPATHIC TYPE 1 DIABETES MELLITUS

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Idiopathic type1 Diabetes Mellitus (DM) in the new ADA's classification is mainly characterised by absence of β-cell autoimmunity in patients with classic clinical criteria. Most of these patients have no HLA association and are of African or Asian origin. Little is known of this type of DM in Caucasian populations. **Aims:** To evaluate the prevalence and differences in clinical presentation and initial evolution of Idiopathic type 1 DM. **Materials and methods:** The study includes 109 consecutive newly diagnosed type 1 diabetes patients admitted in the Endocrine Unit from January 1993 to December 1998, with ages ranging 13-30 years.Patients with classic clinical criteria of type 1 DM and absence of β-cell autoimmunity markers GAD, IA2 were classified as Idiopathic DM. Clinical presentation, insulin-secretory reserves and metabolic control during the first three months after the diagnosis were analysed. Differences between groups were evaluated by student' s T test , the χ 2 test or Mann-Whitney U test. **Results:** Of 109 patients, 87(79.8%) had autoantibodies to islet cell antigens and 22(20.18%) showed negative autoimmunity. Weight loss in Idiopathic DM was less pronounced than in the other group (4.91±4.25 vs 7.59 ±5.26 Kg. p=0.016) and bicarbonate levels at diagnosis were higher (24.03±6.33 vs 19.90±7.06 mmol/L. p=0.016). HbA1c three months after diagnosis was lower in Idiopathic DM patients (4.97±0.64 vs 5.54±1.24 % p=0.007). No significant differences were found in clinical presentation and insulin secretory reserves during the first three months after diagnosis. The percentage of patients with family history of type 2 DM was similar in both groups (27.9% vs 28.57%).**Conclusion**: Idiopathic type 1 DM is present in 20% of our Caucasian population. It is characterised by a less severe clinical presentation and better metabolic control .However, long-term studies and further investigations are necessary to define its etiology and natural history.

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PERSISTENCE OF ISLET CELL AUTOANTIBODIES IN LADA IS RELATED TO SULFONILUREA TREATMENT

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Several experimental studies have demonstrated that sulfonilurea treatment increases autoantigens expression in β-cell. This phenomenon may be deletereous for the preservation of residual β-cell in patients with latent autoimmune diabetes of adult (LADA). The aim of the present study was to evaluate whether sulfonilurea inclusion in the treatment of LADA may induce modifications in the expression of islet cell autoantibodies. Fourteen subjects (mean age of 53 ± 12 years) at diabetes diagnosis were found to be positive for both ICA and GADA in two consecutive samples; ICA were detected by indirect immunofluorescence and GADA by RBA by using recombinant ^{35}S-GAD$_{65}$. Group 1 (n=8, 5 ♂, 3 ♀, age 53 ± 6) was treated with insulin (mean dose 27 ± 20) while group 2 (n=6, 2 ♂, 4 ♀, age 53 ± 16) received together with insulin (mean dose 26 ± 30) 20 mg of glibenclamide. HbA$_{1c}$ decreased in both group after one year of treatment reaching similar levels (6,7% vs 7.0%, p=ns). In group 1, 6 out of 8 patients treated only with insulin became ICA negative, while all 6 individuals treated with the combined therapy presented persistence of ICA titles. No changes were found in GADA in either of both groups, with maintenance of positivity in all patients. Conclusions: addition of sulfonilurea in LADA patients is associated with persistence of islet cell autoantibodies in a higher degree than insulin therapy alone; this may reflect an increased expression of β-cell autoantigens, thereby stimulating autoimmune destruction in this group of patients. (Supported by SAF 97/0251)

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Clinical presentation and early course of type 1 diabetes in patients with a family history of type 2 diabetes.

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Aims: to examine whether a family history of type 2 diabetes can influence the clinical presentation and early course of type 1 diabetes. **Material and methods**: 142 consecutive newly diagnosed type 1 diabetic patients older than 13 years were studied. Subjects were divided into two groups according to the presence (Group I, n=33) or absence (Group II, n=109) of a family history of type 2 diabetes (first degree relatives). Clinical presentation of diabetes, β-cell autoimmune markers (GAD, IA2), thyroid autoimmunity (TPO), evolution of metabolic control and residual insulin secretion during the first year of follow-up, and markers of metabolic syndrome were compared between groups. Statistical analysis was done by Student-t test, Mann Whitney U test or X2 test. **Results**: clinical presentation of diabetes, prevalence of β-cell and thyroid autoimmunity, residual insulin secretion at diagnosis and metabolic control and insulin requirements during follow-up were comparable in both groups. One year after diagnosis, residual insulin secretion (Post-glucagon C-peptide 0.66±0.44 vs 0.45±0.32 nmol/l, p=0.08) and systolic blood pressure (129±12 vs 121±11mmHg, p=0.08) tended to be higher in Group I. LDL-cholesterol was higher (3.2± 0.9 vs 2.7 ±0.7 mmol/l, p=0.03) and HDL-cholesterol lower (1.3 ±0.3 vs 1.5± 0.4mmol/l, p=0.04) in Group I. BMI, diastolic blood pressure, triglycerides and urinary albumin excretion were comparable in both groups. **Conclusions**: type 1 diabetic patients with a family history of type 2 diabetes show no differences in the clinical presentation and early course of the disease. However, they present some markers of the metabolic syndrome. The possible role of these alterations in the apparition or progression of macrovascular complications of the disease awaits further investigations.

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HOW CAN WE IMPROVE THE METABOLIC CONTROL IN ADOLESCENT AND YOUNG ADULT TYPE 1 DIABETIC PATIENTS?

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The transfer of adolescents and young adult with type 1 diabetes mellitus from Pediatric Diabetes Units (PDU) to Adult Diabetes Units (ADU) is frequently a disappointed experience for the patients and their parents. **Aim:** The purpose of this study was to evaluate the impact of this change on metabolic control and treatment aspects of a group of young type 1 diabetic patients. **Patients and methods:** Inclusion criteria were type 1 diabetes initiated in the infancy or adolescence, diagnosed after> 1 y, and with a follow-up of more than 6 months in ADU. Forty-six type 1 diabetic patients (30% woman, age at change 18.5±1.3 y (mean±SD), BMI 21.9±1.9 kg/m^2, diabetes duration 11.9±4.7 y) were selected from the outpatient clinic later than October 1994. **Results:** During the follow-up in ADU (31.0±12.2 months) more patients used intensified insulin therapy (PDU vs. ADU, 9 vs. 56%) and pen injectors (17 vs. 78%, p< 0.05), total NPH insulin were reduced (37.8±12.4 vs. 28.2±9.1 U./day, p< 0.05) as well as the proportion of NPH insulin (75±18 vs. 60±20%, p< 0.05) and NPH doses per day (2.3±0.6 vs. 1.8±0.5/day, p< 0.05). There was a minor increment in BMI (21.7±1.9 vs. 22.5±2.4 kg/m^2, p< 0.05), but no significantive changes in total insulin doses (0.82±0.19 vs. 0.75±0.21 U./kg/day) and insulin injections per day (2.8±1.1 vs. 3.1±0.7/day). Metabolic control improved during the follow-up in ADU (initial, 6, 12, 18, 24 months): HbA$_{1c}$ 9.1±2.0%, 8.6±1.6% *, 8.1±1.5% *, 8.1±1.5% *, and 8.0±1.5% * (* p< 0.05, in relation to basal). **Conclusions:** We conclude that the transfer from PDU to ADU resulted in a major use of regular insulin and pen injectors and a sustained improvement in metabolic control (a HbA$_{1c}$ decrease of ~ 1%) which was evident yet after the first 6 months of follow-up.

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THE METABOLIC AND INSULIN SECRETORY STATUS OF NON-KETOTIC GAD ANTIBODY POSITIVE LATE ONSET DIABETES MELLITUS.

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Antibodies to glutamic acid decarboxylase(GAD) predict early requirement of insulin therapy in patients who present with late onset non-ketotic diabetes mellitus. We studied newly presenting diabetic patients in middle life in an attempt to elucidate their metabolic and immunological status to determine the most appropriate treatment strategy. **METHODS:** 125 sequential newly diagnosed non-ketotic diabetics >35years of age (54.77 [9.25]), males(95 ; 76%), females (30 ; 23%) were challenged with a standardised meal (55% carbohydrate, 30% protein, 15% fat, 550 calories in total) to determine their glucose and insulin responses. The patients were also assessed for the presence of complications in the form of diabetic retinopathy using retinal photography (2x45°fields per eye). The presence of antibodies to glutamic acid decarboxylase(GAD) was determined. The t-test and the Man Whitney test were used to compare data according to whether the variables had a normal Gaussian distribution or not respectively. **RESULTS:** 6 subjects(4.8%) were GAD antibody positive(GAD+ve). Significant differences ($p<0.05$) with GAD antibody negative (GAD-ve) patients include age 48(4.2) vs 55.1(9.3) years, fasting plasma glucose 14(3) vs 10.3(3.2) mmol/l, glucose AUC 0-2hrs (2781.8(391.9) vs 2039.0(543.7) mmol.min/l, HBA1c 9.9(1.4) vs 7.7(2.0)%, and insulin 0-2hrs AUC 422.2(85.65) vs 724.8(332.7) pmol.min/l [Homa beta-cell function 21.1(9.1) vs 51.7(30.18) %] respectively. Non of the GAD+ve patients had evidence of diabetic retinopathy whereas 12.6% of the GAD negative (GAD-ve) subjects had diabetic retinopathy (14 background and 1 pre-proliferative diabetic retinopathy). Two GAD+ve patients (50%) required insulin therapy within 12 month of diagnosis while the other 4 remain on oral therapy with HBA1c >7%(7-9.3%). Only three GAD negative patients (2.5%) required insulin therapy > 2 years after diagnosis. **CONCLUSION:** The presence of GAD antibodies at presentation in patients with late onset diabetes mellitus should alert the need for early insulin therapy based on the severe insulinopenia. Inappropriate initial treatment with oral hypoglycaemic agents may result in prolonged hyperglycaemic exposure and a greater risk of diabetes related complications.

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Diabetes in Chad – high mortality from infectious diseases

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Aim of the study: We evaluated 292 consecutive diabetic patients who were admitted to the General Hospital in Ndjamena/Chad from April 97 to May 98 (age, BMI, complications, symptoms at diagnosis). In 143 patients who were hospitalised, we performed a one year follow-up to evaluate mortality and causes of death.

Results: 65 % of patients (n= 292) were male. The mean age of the patients was 43 years (18 to 70 years). The mean BMI of males was 23 kg/m², females 21 kg/m². The average time since diagnosis of diabetes was 4.5 years. 45 % of the patients were illiterate and 42 % did not have any income. Diabetes was diagnosed in 41.5 % of the patients because they suffered from all of the following symptoms: weight loss, poliuria, polidypsia and poliphagia. In 40 % diabetes was diagnosed because diabetic complications had occurred. At time of diagnosis of diabetes 22.5 % of the patients were hypertensive. 70 % of the patients had diabetic complications (diabetic ulcers in 17 %). 143 patients were hospitalised to improve metabolic control or to treat complications. After 1 year 9 % (of 143) patients had died. Causes of death: infectious diseases: 6 (infected ulcers: 3, AIDS: 2, meningitis: 1). 4 patients died in diabetic coma, 2 from cardiovascular disease, 1 died in renal insufficiency following hypertension).

Summary: In Chad infectious diseases are still a mayor cause of the extremely high mortality in people with diabetes. Instead of spending time to discuss minor changes of WHO diagnostic criteria and classification, developing countries have to concentrate the efforts aiming at improved care for people with diabetes.

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THE IMPACT OF DCCT AND HUMALOG® TREATMENT ON GLYCOHAEMOGLOBIN AND HYPOGLYCAEMIA IN TYPE 1 DIABETES

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Aims: The purpose of this study was to determine if glycohaemoglobin HbA1c values and the incidence of severe hypoglycaemic episodes (seizure, loss of consciousness, or needing the help of others) occurred in young subjects with type 1 diabetes changed from 1993 to 1998. **Materials and Methods:** Data was analyzed from 13580 clinic visits for 950 subjects (460F/490M) with type 1 diabetes for more than one year (mean duration = 9.6 years). All subjects received Humalog insulin for at least one year and had previously received human Regular insulin for at least one year. The mean age was 18.1 years, with 240 < age 10 years, 350 between 10 and 18, and 360 over the age of 18 years when last seen. All HbA1c values were measured using the DCA2000® method. **Results:** Changing to intensive insulin therapy (more than 2 shots/day or insulin pump treatment) reduced the mean HbA1c by 2.4% (95% CI of 1.9 to 2.9%); $p<0.001$ using the mixed effects model but increased the severe hypoglycaemic episodes by 14%/year ($p=0.062$). Introduction of Humalog insulin (mean 2.1 years) resulted in a total reduction of HbA1c by 3.0% (95% CI of 2.5 to 3.4%); $p<0.001$ using the mixed effects model. However a significant decrease in severe hypoglycaemic episodes by 15%/year ($p=0.012$) was observed (adjusted for age and duration). There was no difference in the number of shots/day during the Humalog treatment phase.

Year:	1993	1994	1995	1996	1997	1998
HbA1c	9.4	8.5	8.6	8.6	8.4	8.1
Hypos	0.31	0.36	0.42	0.50	0.47	0.40

Conclusion: A significant decline in HbA1c values with a trend toward an increase in severe hypoglycaemia was observed as subjects were changed to intensive therapy after the DCCT report in 1993. Another significant decline in HbA1c values, accompanied by a significant decrease in severe hypoglycaemia, occurred as subjects were changed to Humalog insulin despite continuing intensive insulin treatment.

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Glucose Sensors and Insulin Infusion Systems

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BLOOD GLUCOSE SELF-MONITORING FROM THE ABDOMINAL SKIN - A VIRTUALLY PAIN-FREE AND PRECISE METHOD

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Aims: For many diabetic patients, years of blood glucose self-monitoring with readings taken several times daily is an inevitable aspect of their insulin therapy. We investigated whether self-monitoring (SM) from abdominal skin might be an alternative to the established method of self-monitoring based on finger-pricking. **Methods:** On 5 successive days, 33 insulin-dependent diabetic patients and 16 nondiabetic volunteers (53% male; aged 46 ± 13.5 years; BMI 25 ± 5 kg/m^2) took parallel blood glucose readings with capillary blood collected from a fingertip and from abdominal skin. Blood was collected from both sites with Softclix II, and readings carried out with an Accutrend Sensor blood glucose monitor. Subsequent enzymatic blood glucose determination in the laboratory (glucose oxidase) from blood obtained by finger-pricking served as reference method. Statistics: Comparison of the methods SM by finger-pricking versus abdominal skin was carried out using Pearson correlations, linear regression analysis, Error-Grid-Analysis and the method after Bland et Altman. **Results:** Self-monitoring from the abdominal skin was found by the trial subjects to cause little discomfort and correlated well with SM and laboratory controls from the finger (Pearson's r 0.88). Comparison of the methods SM abdominal skin versus finger produced results as follows. Linear regression: r = 0.91; y = 19.14 + 0.82x. Error-Grid-Analysis: range A 97.1%; -B 2.5%; -C 0%; -D 0.3%; -E 0.16%. Bland et Altman analysis: mean of the differences 0.16 mmol/l; 2 SD 0.99 mmol/l; minimum – 5.0 mmol/l; maximum 5.1 mmol/l. In a further 5 adipose diabetic patients (BMI mean 32 kg /m ^{2}), SM from abdominal skin was not practicable, as there was insufficient blood to collect. **Conclusions:** SM from abdomal skin is a simple, virtually pain-free and precise method. Following initial quality controls, it provides certain diabetic patients with an alternative to the established method of SM from the fingertip.

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MICRODIALYSIS MEASUREMENT OF GLUCOSE IN SUBCUTANEOUS ADIPOSE TISSUE DURING THREE WEEKS IN TYPE 1 DIABETIC PATIENTS

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Aims: To assess whether microdialysis probes are capable to measure adipose tissue glucose over a prolonged period in Type 1 diabetic patients. **Materials and methods:** Microdialysis probes were pairwise inserted subcutaneously into the abdominal fat and remained in situ for 3 weeks in 8 type 1diabetic patients. At days 1, 3, 4, 8, 11, 16, and 18 of probe retention, glucose as measured by microdialysis was compared to capillary blood glucose concentrations during a 4 h period of normal daily life activities. The recovery glucose obtained by microdialysis, expressed % of the capillary blood glucose concentration, and its variability were evaluated. **Results:** Eleven microdialysis probes in 8 patients were evaluable during the 3 week study period. Recovery of glucose was lower at day 1 and 3 (51 ± 23% and 56 ± 18%, respectively, mean ± SD) compared to values found afterwards (67±19%, 72±13%, 76±14%, 71±16%, and 76±18 %, for day 4, 8, 11, 16, and 18, respectively, for all $P<0.05$ *vs.* day 1 and 3). The mean coefficient of variation of the recovery of glucose measurements was high at day 1 (32±20%) and decreased thereafter to below 15% ($P<0.05$ from day 1). Both the recovery of glucose and its variability did not significantly change after 4 days of in vivo probe insertion. Skinfold thickness was inversely related to the overall glucose recovery (r=-0.76; $P<0.03$). Recovery was similar over a wide range of capillary blood glucose concentrations. **Conclusion.** Prolonged in vivo retention of microdialysis probes improves the recovery and lowers the variability of adipose tissue-sampled glucose in type 1 diabetic patients. These findings give rise to the contention that microdialysis-based glucose measurements offer an opportunity for prolonged glucose monitoring.

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EFFECTS OF GLUCOSE AND INSULIN LEVEL ON ADIPOSE TISSUE GLUCOSE MEASUREMENT BY MICRODIALYSIS PROBES RETAINED FOR 3-WEEKS IN TYPE 1 DIABETIC PATIENTS.

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Aims: To investigate the role of blood glucose and insulin level on the recovery of adipose tissue glucose by microdialysis in vivo.
Materials and methods: Seven Type 1 diabetic patients carried microdialysis probes that were inserted pairwise in abdominal adipose tissue for 3 weeks. A stepped hyperglycaemic-hyperinsulinaemic insulin-clamp was performed each week with blood glucose clamped at 6 and 12 mmol/l, and insulin infused at 30 and 150 mU/kg/h. At each study day, the probes were prepared for microdialysis and samples were collected at 30 min intervals with a microperfusor running at a flow of 0.5 μl/min. Of each probe and of each weekly observation period, the agreement between dialysate, venous and capillary blood glucose levels were analysed.
Results: The overall recovery compared to venous and capillary blood glucose concentrations increased at the 2nd and 3rd week clamps ($P<0.01$ and $P<0.05$ from week 1, respectively). The time-delay to monitor an acute rise in blood glucose ranged from 11.3 to 27.3 min, including a system delay (time to transport fluid from the dialysis membrane to the collection vial) of approximately 12 min. Blood glucose and insulin levels did not influence the recovery compared to venous blood glucose, whereas the recovery compared to capillary blood glucose slightly increased during the high insulin infusion. **Conclusions:** Adipose tissue glucose measurements by microdialysis probes increase after 1 week of insertion. The technique is capable to adequately follow an acute rise in blood glucose although there is still an important time delay inherent to the system. The glucose measurements are not impaired by under hyperglycaemic and hyperinsulinaemic circumstances. These results seem promising for long-term microdialysis in diabetic management.

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NEW GLUCOSE METERS - COMPARISON WITH PREVIOUS GENERATION DEVICES AND A REFERENCE METHOD

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Aims: To assess precision and accuracy of the new portable glucose meters Gluco-Card, Glucometer Esprit, Glucotouch and Glucotrend in comparison to the previous generation meters Accutrend, Companion 2, Glucometer 3 and One Touch II and the reference glucose oxidase method using a Beckman glucose analyzer.
Materials and Methods: On average 248 glucose measurements from capillary blood samples from diabetic patients attending our out-patient clinic were performed with two meters of each brand. Two to three different lots of code strips were used. All measurements were performed by one experienced technician using blood from the same sample for the meters and the Beckman Analyzer 2. Clinically relevant analyses determining the percentage of values within a maximum deviation of 10% and 5% from the reference value, the method of residuals, and the error grid analysis were performed to evaluate accuracy. Coefficients of variance for measurements in series were determined to evaluate precision.
Results: Altogether 1,987 blood glucose values were obtained for readings with meters compared with the reference method. By error grid analysis the new devices gave more accurate results without significant differences within the group (zone A: 98 to 98.5%). Except the One Touch II (zone A: 98.5%) the other older devices were substantially less exact (zone A: 87 to 92.5%), which was also true for all other evaluation procedures. With respect to a 10% deviation from the reference values the Glucometer Esprit was inferior to all other new meters (81% vs. 88.5 to 90.5%), but similar to the One Touch II which performed best compared to the other old generation meters (82% vs. 56 to 72%). Less than 5% deviation from reference values was found in 49 to 57% for new, and in 32 to 50% for old devices. Companion 2 underestimated, Glucometer 3 overestimated reference values by more than 10%. With coefficients of variance higher than 10% in low glucose ranges precision was worst for Companion 2 and Glucometer Esprit.
Conclusions: New generation blood glucose meters are not only smaller and handsomer but more accurate compared to previous generation devices except the One Touch II. Clinical performance of new meters improved but did not reach latest recommendations of the American Diabetes Association.

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ANTIGENICITY OF HOECHST 21PH INSULIN USING INTRAPERITONEAL OR SUBCUTANEOUS ROUTES IN TYPE 1 DIABETIC PATIENTS.

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Intraperitoneal (IP) administration of Hoechst21PH insulin (400U/ml) via implantable devices increases insulin antibody levels (AAI) in type 1 diabetic patients. Many factors may be involved in this process ; IP route of administration, addition of a stabilizer (Genapol°) to the insulin solution or insulin modifications due to storage in the pump. **Aims :** to compare the antigenicity of the insulin Hoe21PH administered intraperitoneally or subcutaneously using implantable or external pumps. **Material and methods :** Hoe21PH was used at a concentration of 400U/ml in the Minimed MIP pump and 100U/ml in the H–tron (Disetronic) external pump. The 19 type 1 diabetic patients had been treated with human insulin for 3 years prior to the study and were then treated either with IP infusion (CPII) (n=10, group 1) or subcutaneous infusion (CSII) (n=8, group 2). AAI levels were assessed by RIA (Pasteur, N< 2.5%) and Elisa (N<12 U/ml) at the beginning of the study (M0) and after 6 months (M6). **Results** (mean ± SEM) **:** Patients were 35.0±1.8 vs 34.9±4.9 year old, had a diabetes duration of 17.3±2.1 vs 30.1±4.8 years for respectively group 1 and 2. Sex ratio M/F was 6/4 vs 5/3. AAI levels (RIA) increased from 21.2 ± 8.5% (M0) to 38.8 ± 7.4% (M6) in group 1 (p=0.04) and from 31.1 ± 11.3% (M0) to 34.9 ± 11.3% (M6) in group 2 (p=0.95), using a matched Student t test. Evolution of AAI formation tended to be higher in group 1 than in group 2 (p=0.08) using an ANOVA test. The same tendency was observed using the Elisa assay. CPII using implantable devices seems to induce higher levels of AAI than CSII. Factors correlated to high AAI formation would rather explain increased AAI levels in group 2. **Conclusion :** CPII tends to be more antigenic than CSII. Insulin modifications due to insulin storage in external or implantable pump could be also involved in this high antigenicity.

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CONTINUOUS INTRAPERITONEAL INSULIN INFUSION (CIPII) IN POORLY CONTROLLED PATIENTS: GLYCAEMIC RESULTS AND PSYCHOSOCIAL CHARACTERISTICS

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Aims: To investigate retrospectively the glycaemic efficacy of CIPII (implantable pump) in patients in long-term poor control, as well as predictors of success, quality of life and emotional well-being. **Methods:** data of 33 patients were recorded on standardized forms by the treating physician. Patients filled in questionnaires on sociodemographic characteristics and psychopathology (as possible determinants of persistent poor control) (SCL-90), quality of life (Diabetes Quality of Life Measure, SF-36) and emotional well-being (Well-being Questionnaire, Problem Areas In Diabetes). **Results:** Patient characteristics. M/F: 9/24, age: 42.6 (SD 13.3), type 1: 84.8%, diabetes duration: 20.5 ± 8.4 years. 51.5 % is unemployed. 15 % is free of long-term diabetic complications. Mean HbA1c decreased in the 24 patients implanted because of poor control (HbA1c before CIPII ≥ 8.5%), from 10.8% before implantation to 9.3% in 1998, mean follow-up 63 months (range: 24-168) (P= 0.006). There were no indications of severe psychopathology in this group. However, levels of quality of life, social functioning, and well-being were found to be low, while levels of diabetes related problems were high. Responders (experiencing a decrease of > 1% HbA1c on CIPII, n=10 of 24) had higher HbA1c levels at baseline (P=0.013), and lower levels of social (P=0.02) and physical functioning (P=0.001). **Conclusions:** Our results suggest that CIPII can be valuable for patients in persistent poor glycaemic control, even in patients with low levels of physical and social functioning. Prospective trials are required to confirm these findings.

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Continuous Subcutaneous Insulin Infusion for Treatment of Type 2 Diabetes Mellitus.

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Aims: The utility of continuous subcutaneous insulin infusion (CSII) for treating type 1 diabetes has been clearly demonstrated. The benefit of normoglycemic control of persons with type 2 diabetes is established, but often requires increasingly complex regimens of combination therapy that are costly and introduce potentially toxic interactions of multiple diabetes related drugs. CSII therapy may offer an effective alternative.

Methods:. We have reviewed all type 2 diabetes patients from our large database of CSII patients in order to evaluate CSII as an alternative method of treating type 2 diabetes. Eleven patients were found to be C-peptide positive and/or to have a history of several years of no insulin therapy since diagnosis.

Results: All patients had been converted to CSII after failure to be adequately controlled on multiple daily injections (MDI). The patients were 55 ± 12 years old with diabetes for 13 ± 6 years, and performed self monitoring of blood glucose a median of 3.05 times per day. The average pre-CSII HbA1c was 9.3 ± 2.8 which decreased to 6.97 ± 0.9 at 18 months (*p=0.046*). The daily insulin requirement decreased from 89 ± 62 to 70 ± 53 units/day at 6 months (*p=0.011*). The patients weight did not change significantly from pre-CSII to 18 months (190 ± 53 vs 191 ± 52 lbs). No severe hypoglycemia was documented in 18 months follow-up.

Conclusion: This limited experience in using CSII in patients with type 2 diabetes suggests that CSII appears to provide a level of improved clinical outcomes comparable to that experienced by type 1 patients. CSII should be considered as a viable alternative therapy for the treatment of type 2 diabetes.

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CONTINUOUS SUBCUTANEOUS INSULIN INFUSION (CSII) IN NEONATAL DIABETES MELLITUS

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Neonatal diabetes mellitus is extremely rare (1/400 000 births in Europe). Typically, infants are of low birth weight and develop hyperglycemia requiring exogenous insulin within the first six postnatal weeks. Although pediatricians face numerous difficulties in managing insulin therapy (IT) at this age, very few data are available on the methods of insulin delivery in neonatal diabetes. We report our experience of CSII therapy in the cases of neonatal diabetes requiring subcutaneous insulin therapy for more than 15 days (n=4), that we have treated during the last 10 years. Three of them were transient diabetes (follow-up = 10 months to 6 years) and one was permanent.

Case	Birthweight (g) and term (weeks)	T0 insulin/ CSII (age in days)	Total daily dose (TDD, U/kg/day) / Daily basal rate (% of TDD)			Duration of IT
			T0 CSII	+15 days	+1month	
1	2530g(38w)	D2 / D7	0.57 / 100	0.96 / 100	0.45 / 100	2.5mo.
2	1430g(38w)	D15 / D17	0.70 / 100	0.29 / 100	0.40 / 36	7 mo.
3	1200g(33w)	D21 / D33	0.30 / 100	0.90 / 100	0.60 / 30	9.5 mo.
4	2100g(38w)	D42 / D63	0.73 / 42	1.0 / 40	0.65 / 35	9 yrs

During the first month of CSII therapy, mean blood glucose level was 171mg/dl and mean number of hypoglycemia (<60mg/dl) was 5.7 on a mean number of 266 blood glucose measurements. CSII therapy in neonatal diabetes allowed to meet the specific insulin requirements and to adapt to the conditions of nutrition: basal rate only (100% of TDD) under continuous enteral or parenteral feeding; preprandial boluses were started with feeding bottles. Management of very small insulin doses (ex. bolus = 0.12 U and basal rate = 0.018 U/h) was possible after dilution (5-10U/ml) and was more accurate with CSII than with syringes. Controlling blood glucose with few hypoglycemias, which are particularly frequent and dangerous at this age, was more efficient with CSII than with injections. Cutaneous tolerance was good and we did not observe any side effects. For all children, CSII was carried on during the whole period of insulin therapy (2.5 months to 9 years). Conclusions: during the neonatal period, CSII therapy is safe, more physiological, more accurate and easier to manage than injections and allows to match the insulin requirements of a newborn. CSII therapy requires to be managed or supervised by an experienced team.

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INITIATION OF INSULIN PUMP THERAPY: THE OUT-PATIENT PUMPSCHOOL VS. IN-PATIENT HOSPITAL INITIATION.

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Aims: To compare the efficacy, patient (pt) competency and discontinuation rate of out-patient (outpt) vs. in-patient (inpt) insulin pump therapy (IPT) initiation. **Materials and Methods:** Successful IPT initiation requires pt readiness, diabetes management skills, a conducive learning environment, and experienced team, with 24-h patient access. Hitherto, a specialized inpt unit best met the education/safety needs. We developed an outpt model, the PumpSchool (PS) and compared outcomes with the prior traditional 2-5 day inpt initiation. We retrospectively compared the results of the first 59 PS pts (mean age38.6±16; 33 males) with the antecedent 59 inpts (mean age 39.6±12; 24 males). All pts elected IPT to improve glycemic control/flexibility after failing multiple daily insulin injections. The PS was held Thursday/ Friday from 0830-1700h; pts assumed responsibility for overnight blood glucose (BG) monitoring. PS pts arrived fasting; the nurse practitioner inserted a Rapid infusion set (Disetronics) s.c. in the abdomen, with prior programming of the Disetronic H-Tron. BG monitoring was done: pre- and 1h post-meal, bedtime, 0200h, 0400h and if symptomatic, with ongoing basal and bolus adjustments. Meals were based on carbohydrate (CHO) distribution of pt's prior plan; all food was weighed/measured. Initial insulin dose was 80% pre-IPT total: ≅ 50% as multiple basals; ≅ 50% titrated to CHO g planned per meal. Pts used an individualized insulin algorithm for hyperglycemia. Interactive multi-media group teaching was conducted for 8 h/day. By end of Day 2, all pts were competent with acceptable glucose control. **Results: (a)** % pts meeting American Diabetes Association (ADA) goal HbA1c ≤ 1% upper limit of normal: PS pts: pre-IPT, 45.8%; post-IPT, 67.8%; Inpts: pre-IPT, 35.6%; post-IPT, 59.3%; **(b)** both PS pts and Inpts were competent in pump use by training end; **(c)** discontinuation rate: PS pts, 8.5%; Inpts, 11.9%; reasons: infusion set pain, pump encumbrance; **(d) Conclusion:** As compared to the more costly inpt setting, IPT initiation in a dedicated PS is equally successful, with more pts meeting ADA goals and high pt competency/low discontinuation rates.

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DO TURKISH IMMIGRANTS WITH DIABETES MODIFY THEIR NUTRITIONAL HABITS WHEN THEY ARE LIVING IN GERMANY?

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Aims: It is assumed that Turkish immigrants with diabetes who live in Germany will adapt their life-style to local habits. We investigated, whether people with diabetes living in Istanbul (n=50) and Turkish immigrants with diabetes living in Düsseldorf (n=50) differ in their nutritional habits and well-being. **Materials and Methods:** Standardized questionnaires in Turkish language were administered to all individuals by one interviewer (Structured Nutritional History: Toeller 1995, Adjective Mood Scale Bf-S'and List of Complaints B-L : Zerssen 1976). **Results:** No significant differences were observed in physical and mental well-being (Bf- S'sumscores: 16.1±8.0 and 15.4±9 2 and B-L'sumscores: 16.6±9.2 and 16.2±10.2), however, HbA_{1c}-, Triglycerides-, Cholesterol- and LDL-Cholesterol were higher in Turkish immigrants from Düsseldorf as compared to persons with diabetes living in Istanbul. Turkish immigrants living in Düsseldorf consumed more frequently ($p<0,05$) bread with butter, margarine, sausages, cheese and jam as well as special diabetic products, which are not commonly used in Turkey. Nevertheless, 36 (72%) of the 50 Turkish immigrants, who lived in Germany on average for 25 years, felt that they had not changed their nutritional habits after leaving Turkey. **Conclusions:** We conclude that overtime Turkish immigrants with diabetes living in Germany unconsciously adopt some of the typical German eating habits. It is, however, not clear whether these life-style modifications contributed to poorer control seen in the Turkish immigrants. No differences between the two groups with regard to physical and mental well-being could be observed.

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ENERGY EXPENDITURE DURING ORAL GLUCOSE LOAD IN TEN UREMIC PATIENTS BEFORE AND AFTER THREE MONTHS ON A LOW PROTEIN DIET.

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Aims : we have previously shown that very low protein diet (VLPD) which has been proposed to slow down the rate of progression of chronic renal failure (CRF), improved tissue insulin sensitivity and decreased hyperinsulinemia in predialytic uremic patients. However such diet prescription may potentially result in denutrition. Materials and Methods : The purpose of the present study is to study basal energy expenditure and energy expenditure (EE) during an oral glucose load in CRF patients on VLPD ($0.3.kg\ wt^{-1}.day^{-1}$) using oral glucose load in combination with indirect calorimetry. We also asses body weight and analyse body composition by DEXA during VLPD. Results : After three months on VLPD, no significant change in total body weight was observed but DEXA showed a decrease of lean tissue mass (46.2 ± 3.6 before versus 44 ± 3.4 kg after 3 months of VLPD ; $p<0.01$) and a rise in body fat mass (20.1 ± 2.4 before versus 21.3 ± 2.4 kg on VLPD ; $p<0.05$). In the post absorptive state plasma glucose was significantly lower and glucose oxidation and energy expenditure per lean tissue mass (LTM) were significantly increased (EE :20 ± 0.8 kcal. kg $LTM^{-1}.min^{-1}$ before diet versus 21.9 ± 1.1 kcal. kg $LTM^{-1}.min^{-1}$ after 3 months on VLPD, $p<0.01$). In the feeding state, plasma glucose and insulinemia were significantly lower whereas glucose oxidation rose. EE values were significantly higher during the oral glucose load from the 30^{th} to the 270^{th} min (at t=270 min, 20.1 ± 0.9 kcal. kg $LTM^{-1}.min^{-1}$ before the diet versus 23.1 ± 1.1 kcal. kg $LTM^{-1}.min^{-1}$ after 3 months of VLPD, $p<0.001$). Glucose oxidation was higher and cumulated glucose storage was decreased after diet : 29.6 ± 4.2 g versus 20.9 ± 3.4 g on VLPD ($p<0.01$). Conclusion : After 3 months on VLPD, total body weight was not changed but lean tissue mass decreased. VLPD increases EE in the post absorptive state and during an oral glucose load. During VLPD a strict survey of dietetic management is necessary to maintain energy requirements at high levels to prevent malnutrition that may be an adverse effect of VLPD.

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REGULATION OF LEPTIN BY DIETARY POLYUNSATURATED N-3 AND MONOUNSATURATED FATTY ACIDS IN SUCROSE-FED RATS.

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The aim of the present study was to evaluate the relationship between leptin and insulin-resistance during dietary manipulation of different fatty acids: polyunsaturated (n-3) or monounsaturated fatty acids in a rat model of insulin-resistance, the sucrose-fed rat. **Materials and Methods:**Sixty-four male Sprague Dawley rats were randomized into four groups. Three groups were submitted for 3 or 6 weeks to a diet containing 57% sucrose and 14% lipids as either fish oil (SF) , olive oil (SO) or a standard mixture of vegetable and animal oils (SC). A fourth, reference group (R), was submitted to a diet containing 57% starch and 5,5% lipids as a standard mixture of vegetable and animal oils. **Results:**After 3 and 6 weeks sucrose-fed rats (SC vs R) were characterized by a slight hyperglycemia, hypertriglyceridemia and by low Glut-4 protein quantity in adipocytes. Three weeks of sucrose diet induced hyperinsulinemia wich was not observed at 6 weeks. Changing the type of lipid in the sucrose diet for either 3 or 6 weeks had no effect on either food intake, body weight or total fat mass in the SF fed rats. Both epididymal and retroperitoneal adipose tissue relative weights were lower in the SF group than in the SC and SO groups ($p< 0,005$, ANOVA). Fish oil prevented also the hyperinsulinemia (3 weeks), hypertriglyceridemia and the decrease in the quantity of Glut-4 protein found in the SC group. These effects were associated, at any time, with an increase in plasma leptin level and ob gene expression in retroperitoneal adipose tissue. Olive oil, however, was not able to prevent neither the sucrose-induced insulin resistance (estimated by hyperinsulinemia, hyperglycemia, hypertriglyceridemia, decreased Glut-4), nor the sucrose-induced adiposity. Although insulin-resistance did not change, plasma leptin was found to be increased without any change in food intake. **Conclusions:** in the sucrose-fed insulin-resistant rats, the presence of fish oil prevented insulin resistance, adiposity and increased leptin levels. The presence of olive oil could neither ameliorate insulin-resistance nor adiposity but was associated with high leptin levels after long-term diet. These results suggest that poly- and monounsaturated fatty acids regulate differently insulin-resistance and leptin levels. The relation between leptin and insulin-resistance is still complex and not well definied.

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EFFECT OF VITAMIN E ON GLUCOSE TOLERANCE AND LIPID PEROXIDATION IN DEXAMETHAZONE-TREATED RATS

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There is increasing evidence that oxidative stress plays a causative role in impairment of insulin action. **The aim** of the study was to evaluate impact of vitamin E (E) on glucose tolerance and lipid peroxidation in rats with dexamethazone (D)-induced insulin resistance. **Materials and methods:** Male Wistar rats (3-mo-old) were injected D (0.125 mg/kg s.c. 13 days). Control rats (C) were given vehicle alone. One group of D-treated rats received E (100 mg/kg per os) starting 4 days after the first D-injection and another group was given placebo to act as D control (DP). At the end of the study fasted rats were subjected to a glucose tolerance test (GTT, 2 g/kg i.p.). Lipid peroxidation was estimated in plasma by determination of malonic dialdehyde (MDA) and diene conjugates (DC) contents. **Results:** At day 14 after the first D-injection there were no differences in basal blood glucose levels between all experimental groups. However, glucose tolerance was impaired by D-administration (integral glycemia over GTT was 44.1±1.1 vs C: 21.6±1.2 mmol/l, $p<0.01$). E prevented development of D-induced glucose intolerance reducing integral glycemia 2-fold ($p<0.01$) in comparison with D-injected control rats. Moreover, E-supplementation attenuated lipid peroxidation decreasing DC contents (0.59±0.03 vs DP: 0.93±0.05, $p<0.05$; C: 0.68±0.04 μmol/ml) and MDA concentration by 50 % ($p<0.02$) compared to D-treated control group. **Conclusions:** These data suggest that E reduces lipid peroxidation and prevents impairment of glucose tolerance in dexamethazone-treated rats. Thus, vitamin E may have potential beneficial effect in the treatment of NIDDM.

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CARBOHYDRATE SUPPLYING FOOD GROUPS DIFFER IN THEIR RELATION TO HbA_{1c} IN PERSONS WITH TYPE 1 DIABETES

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Aims: More information is needed on the relationship between glycated hemoglobin levels and the source or amount of dietary carbohydrate. The present study compares the association of carbohydrate intake with HbA_{1c} between European individuals with type 1 diabetes injecting insulin 1 or 2x/day and those with ≥3 daily injections. **Materials and Methods:** The relation of carbohydrate intake (total, cereal, fruit, vegetable, milk, and potatoe carbohydrate assessed by a 3-day dietary record) to HbA_{1c} was examined in 2084 patients (age 32.6±10.2 years, diabetes duration 14.8±9.5 years). **Results:** In individuals injecting insulin 1 or 2x/day increased intakes of total carbohydrate were related independently to higher HbA_{1c} concentrations ($p=0.005$); an increased consumption of potatoe and milk carbohydrate was associated with higher levels of HbA_{1c} ($p=0.004$ and $p=0.0001$, respectively), whereas an increased intake of vegetable carbohydrate was inversely related to HbA_{1c} ($p=0.002$) and for cereal and fruit carbohydrate no significant associations were seen to HbA_{1c}. In individuals injecting insulin ≥3x/day HbA_{1c} was related independently to intakes of potatoe carbohydrate ($p=0.01$), but not to the other carbohydrate sources. **Conclusions:** Subjects with only 1 or 2 daily insulin injections need specific education with regard to the consideration of larger amounts of milk and potatoe carbohydrate, however, they may profit from a higher vegetable carbohydrate consumption. In individuals with type 1 diabetes injecting insulin ≥3x/day the type or amount of dietary carbohydrate seems to have little influence on their long-term glycemic control.

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CHRONIC EFFECTS OF A LOW GLYCEMIC INDEX BREAKFAST ON GLUCOSE AND LIPID MEATBOLISM IN NIDDM MEN.

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Although the fact that the ingestion of equal amonts of different starches can lead to variations in subsequent plasma glucose concentrations, the clinical utility of these findings for treatment of NIDDM patients is questionned. **Aim** : The aim of the present study was to evaluate whether the chronic use of low glycemic index breakfast could ameliorate glucose and lipid metabolism in NIDDM men. **Materials and Methods** : Thirteen NIDDM men participated in this study (fasting glycemia 10.8 ± 2.8, HbA1c 8.5 ± 1.6). The patients were randomly allocated in a cross over design to two periods of 4 weeks of daily intake of a breakfast rich in low glycemic (pumpernickel, cereal based on extruded oat bran concentrate, apple and fructose LGI-B) or high glycemic index (wholemeal bread, weetabix, HGI-B) starch. **Results** : At the end of 4 weeks of either LGI-B or the HGI-B results of the 7-days records demonstrated that the daily intakes of total energy and macronutrients were unchanged. Body weight was also comparable after LGI-B or HGI-B. The LGI-B showed lower postprandial plasma glucose peaks than the HGI-B at the beginning (Day0) and after the 4 weeks of chronic intake. Fasting plasma glucose, insulin or HbA1c were not affected by the chronic intake. Fasting plasma cholesterol ($p<0.05$) (total, esterified and free) as well as the surface under the cholesterol curve after both diets were lower after 4 weeks of the LGI-B than after the HGI-B ($p<0.05$). The ApoB showed also lower values after the 4 weeks LGI-B than after 4 weeks of the HGI-B ($p<0.03$). **Conclusion** : We demonstrated that a low glycemic index breakfast inducing low glycemic postprandial peaks when taken for 4 weeks could decrease plasma cholesterol levels which is one of the main cardiovascular risk factors.

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GLYCEMIC AND INSULINEMIC INDEX OF MANGO AND PAPAYA IN TYPE 2 DIABETIC SUBJECTS

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Aims: Glycemic Index (GI) and Insulin Index (II) are useful measures of serum glucose and insulin responses to a dietary component. The GI and II of foods may be variable in different regions and races. Accordingly, local food items need to be ranked on the basis of their GI and II. This study was undertaken to determine the GI and II of Mango and Papaya from Bangladesh origin with white bread as the reference food. **Materials and Methods:** Thirteen subjects (male 7 and female 6) participated in the study, under a cross-over design, in which they consumed equi-carbohydrate amount of the fruits and bread, with a run-in period of 5 days between the consecutive items. The mean (±SD) age (years) of the patients was 39.46±6.21 and they had a basal serum fructosamine value of 391±93 µmol/l. Serum glucose was measured by glucose-oxidase method and fructosamine was measured by colorimetric method. Serum C-peptide was used as the marker of insulin and it was measured by an ELISA technique. **Results:** Mango and Papaya showed higher serum glucose responses compared to that of bread (Area under the curve: 2153±566 in Papaya and 2138±639 in Mango vs 1745±450 in Bread; p <0.072 and 0.091 respectively), but there was no difference between Papaya and Mango. The similar glycemic responses of Papaya and Mango were reflected in their GI values (Papaya 124±14 and Mango 122±10, M±SD). In contrast to glycemic response Papaya showed higher insulin response compared to both Mango and Bread [Absolute increment in C-peptide: Median (Range); Papaya - 2.732 (0.65-5.0), Mango - 1.05 (0.21-0.95) and Bread - 2.73 (0.65-5.0); p<0.001 for both fruits compared to Bread]. The higher insulinemic response of Papaya was reflected in the significantly higher C-peptide – glucose ratio of the fruit in comparison to that of Mango and Bread [C-peptide - Glucose ratio (M±SD): Papaya - 0.52±0.25; Mango - 0.39±0.21 and Bread 0.44±0.21; p< 0.010 and 0.003 respectively]. **Conclusions:** The data suggest the following: a) Equi-carbohydrate amount of Papaya and Mango produce higher glycemic response as compared to bread, b) From the view point of blood glucose control the two fruits seem to be interchangeable, but higher insulin response of papaya needs to be considered in case of therapeutic management of diabetic patients and in assessing the risk of atherogenesis due to hyperinsulinemia.

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OXIDATIVE DNA DAMAGE IN DIABETES MELLITUS AND THE EFFECT OF VITAMIN E.

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Aims: The aim of this study was to establish the oxidative DNA damage which a marker of oxidative stress in diabetic patients and the effect of Vitamin E on oxidative DNA damage.

Materials and Methods: To test whether DNA is oxidatively damaged in Diabetes Mellitus (DM), we have evaluated the DNA damage by Alkaline Single Cell Gel Electrophoresis (SCGE) technnique (comet assay) in lymphocytes of 63 diabetic patients (15 Type I DM and 48 Type II DM, 36 female and 27 male) and of 30 sex and age matched healthy control subjects. Serum Total Antioxidant Status (TAS) and Malonyl Dialdehyd (MDA) level which are indicators of oxidative stress were also measured in patient and control group. The patients were randomised into two groups given vitamin E (900mg/day) and plasebo for 12 weeks.

Results: The mean SCGE of damaged cells and serum MDA level was higher in diabetic patients than control group (SCGE: 38.10±19.50 and 11.05±6.05, 138.38±45.22 nmol/kg and 60.28±10.62 nmol/kg respectively, p<0.005) and serum TAS level is lower (1.47±0.27 mmol/l and 1.75±0.30 mmol/l, p<0.05). We determined more significant reduction in oxidative DNA damage in patients who used vitamin E than the patients who used placebo (from 33.47±19.95 to 19.88±11.22, p<0.005 vs. from 43.79±17.72 to 42.54±18.62, p<0.05 respectively).

Conclusions: Using vitamin E in diabetic patients as an antioxidant therapy can decrease oxidative DNA damage and this may reduce the frequency of diabetic complications.

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ORLISTAT-INDUCED WEIGHT LOSS IMPROVES INSULIN RESISTANCE IN OBESE PATIENTS

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Aims: To investigate the effects of weight loss promoted by orlistat, a novel non-systemically acting anti-obesity agent, on insulin resistance in obese patients. **Materials and Methods:** Data from 5 multicentre, 2-year, randomised, placebo-controlled trials of similar design were pooled. All studies involved a 4-week single blind placebo lead-in during which obese patients (BMI 28-43 kg/m^2) received a mildly hypocaloric diet (500-800 kcal daily deficit). Patients were stratified according to diet-induced weight loss (≤2 kg or >2 kg) and randomised to treatment with orlistat 120 mg tid (n=1561) or placebo (n=1119). All patients continued on the hypocaloric diet for the first year of treatment, and switched to a weight maintenance diet in the second year. Fasting glucose and insulin values were monitored frequently and used to measure insulin resistance index, using the homeostasis model assessment (HOMA-R), calculated as insulin (pmol/L) × glucose (mmol/L)/22.5. **Results:** Mean weight loss after 1 year was significantly greater in the orlistat group (9.2% vs 5.8% for placebo; p<0.001). Orlistat-treated patients had significantly greater improvements in fasting glucose (mean change from baseline: placebo 0.00 ± 0.02, orlistat -0.04 ± 0.02mmol/L, p=0.001) and insulin (placebo 5.16 ± 3.48; orlistat –6.69 ± 3.01 pmol/L p<0.001) after 1 year of treatment. Furthermore, orlistat-induced weight loss was accompanied by significantly greater improvements in insulin resistance index (least squares mean [lsm] difference from placebo = -0.51; p=0.003). Greater weight loss with orlistat was maintained after 2 years of treatment (6.7% vs 3.7%; p<0.001) and this was also associated with significant improvements in insulin resistance (lsm difference from placebo = -0.84; p<0.001). **Conclusion:** Orlistat-induced weight loss is associated with significant improvements in insulin resistance.

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FRUCTOSE DIET INDUCED INSULIN RESISTANCE CAUSES SELECTIVE SYMPATHETIC STIMULATION: CORRECTION BY METFORMIN

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Aims: The aim of the present study was to investigate the effect of high fructose diet on sympathetic nervous system (SNS) activity. **Materials and Methods:** In a first step, Sprague-Dawley male rat were fed for 4 weeks either on a standard diet (C group) or on a high-fructose diet (F group, 10 % in drinking water). After two and four weeks, hyperinsulinemic-euglycemic clamps (6 mU insulin/kg.min) were performed on awake unrestrained rats to test insulin resistance. This enabled us to conclude that at least one month of fructose diet is required to induce reproducible insulin resistance (33.6±1.5 C vs 22.8±2.2 mg glucose/kg.min F, p<0.05). In a second step, SNS activity was measured in control (C) and one month fructose diet (F) fed rats, in each group half of the animals received metformin in drinking water for 4 weeks (500 mg/kg.day, C+M and F+M). Ten min. before sacrifice, rats received an i.p. injection of NSD (m-hydroxybenzylhydrazine, inhibitor of DOPA decarboxylase, 100 mg/kg), DOPA accumulation was used as an index of SNS activity and measured in superior cervical and coeliac ganglias. **Results:** SNS was increased in F group only in coeliac ganglia (16.8±1.1 C vs 22.6±2.2 ngDOPA/ganglia, F group, p<0,05) and not in superior cervical ganglia (8.4±0.7 C vs 8.6±0.7 ngDOPA/ganglia, F group, ns). Metformin treatment restaured insulin sensitivity (33.6±1.5 C vs 30.9±3.1 mg glucose/kg.min F+M, ns). Metformin had no effect on SNS acitivity of control animals (15.9±1.7 C+M vs16.8±1.1ng DOPA/coeliac ganglia C, ns) but prevented the increase in SNS activity in fructose fed animals (22.6±2.2 F vs 16.3±2.8 ng DOPA/coeliac ganglia F+M). **Conclusion:** our results demonstrate that one month of fructose diet is required to induce insulin resistance. Fructose diet caused a selective increase in SNS activity. Both effects, i.e. insulin resistance and increased SNS activity are prevented by metformin treatment.

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Islet and Pancreas Transplantation

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DETECTION AND SEMI-QUANTIFICATION OF PORCINE ENDOGENOUS TYPE C RETROVIRAL mRNAS IN DIFFERENT TISSUES FROM SPF PIGS

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Aim : Pigs could be donors for islet grafts in human type I diabetes. One difficulty to be solved before such xenograft can be used is the risk of transmitting infectious agents. This led us to use specific pathogen-free (SPF) pigs (collaboration with CNEVA, Ploufragan, France), a practice which offers protection against the risk of " conventional " xenosis in the context of a strategy of quality assurance and sanitary control. However, the use of SPF pigs may not change the risk of transmitting porcine endogenous retroviral sequences (PERV). As the " retroviral " risk may depend on the type of tissue intended to be grafted, we studied the presence, and compared the expression of PERV mRNAs in different SPF pig tissues. **Materials and Methods** : Frozen samples of 14 different tissues were obtained from 6 SPF pigs. " Semi-quantitative " RT-PCR (with beta-actin mRNA as " internal standard ") was performed to detect - and measure the level of - mRNAs for "gag", "pol", and two "env" subtypes (A and B). Autoradiographs were obtained and the intensity of the bands was quantified by densitometry. Results expressed as mean values ± SEM were statistically compared using Student's t-test. **Results**: Retroviral mRNAs for "gag", "pol", env-A and env-B, were present in all tissues studied from the 6 SPF pigs. However, the level of expression varied between tissues. Among tissues which are intended to be grafted in man, the mRNA levels were lower ($p< 0.01$) in pancreas than in the others tissues (kidney, lung, heart and liver). "Pol" mRNA was twenty-two times less abundant in pancreas than in the retroviral-producing porcine cell line G2. **Conclusions** : As this " semi-quantitative " study was analysed using beta-actin as " housekeeping " gene which is known to display somewhat variable expression, it appears necessary to confirm the results with other " internal standards " (GAPDH, HPRT...). However, mRNAs for three type C retroviral genes were detected in all SPF pig tissues. This preliminary study suggests that levels are variable between tissues, with the lower level detected in pancreas. Retroviral mRNAs were much less abundant in SPF pig tissues than in a retroviral-producing porcine cell line.

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IN VITRO RECOGNITION AND AGGRESSION OF PIG ISLETS BY HUMAN MONONUCLEAR CELLS. WHICH IMMUNOSUPPRESSIVE AGENTS ?

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Aims: To investigate human cellular rejection of pig islets, we previously described *in vitro* the mechanisms involved in Peripheral Blood Mononuclear Cells (PBMC) proliferation in response to pig islet cells. We attempted here to determine immunosuppressive agents that could reduce this strong cellular reaction. **Materials and methods**: 500000 human PBMC were incubated during 7 days with 60000 porcine islet cells; lymphocyte proliferation was determined by 3H-thymidine uptake. As lymphocyte proliferation is not indicative of aggression against islet cells, we also measured for the first time dynamic basal and stimulated insulin release, in response to glucose, from pig islet cells co-incubated with human PBMC. We studied the ability of cyclosporine A (CsA), mycophenolate and gadolinium to reduce proliferation and aggression of islets by incubating immunosuppressive agents 2 hours with human PBMC before co-culture. **Results:** Cyclosporine A (CsA) and mycophenolate dose-dependently inhibited human PBMC proliferation induced by pig islet cells (74%±9 and 100% inhibition for 1µg/ml CsA and mycophenolate, respectively; $p<0.03$). Gadolinium also inhibited this proliferation, (78%±21 inhibition for 500µg/ml; $p<0.05$). After 7 days of co-culture with human PBMC, both basal and stimulated insulin releases of pig islet cells were strongly and irreversibly decreased ($p<0.001$). Insulin release was also decreased after co-culture of islets with purified human Antigen Presenting Cells (APC), whereas depletion of APC from PBMC almost completely abolished inhibition. CsA and mycophenolate (1µg/ml and 0,5µg/ml respectively) had no effect on inhibition of insulin release by PBMC, whereas gadolinium (100µg/ml) almost completely abolished this inhibition. **Conclusions:** The test based on inhibition of insulin release indicates that cellular aggression against pig islet cells results in a first step from toxic effects induced by APCs. Therefore, immnusuppressive agents such as CsA and mycophenolate, which only interfere with proliferation of activated T lymphocytes, cannot inhibit this first step of cellular xenogeneic rejection. By contrast, gadolinium, which inhibits macrophages, prevents this first toxic step and also blocks presentation of xenoantigens by APCs leading to inhibition of T lymphocyte proliferation.

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IMMOBILIZATION OF PANCREATIC ISLET CELLS ON A TWO-DIMENSIONAL MICROSUPPORT

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Aims: Immobilized whole cells with preserved viability represent a useful tool for biotechnological applications, e.g. transplantation. The present work introduces a new method for the immobilization of pancreatic islet cells to a two-dimensional microsupport. **Methods:** Tumoral islet cells of the RINm5F line were cultured for 24 h in the presence of two-dimensional microsupports ($0.7.10^6$ cells/ml). The metabolism of D-glucose (2.8 mmol/l) was then measured over 120 min incubation at 37°C in a bicarbonate-buffered medium containing 5 mg/ml bovine serum albumin. **Results:** Less than 10 % of the cells initially added failed to be immobilized. The protein (about 0.1 ng/cell) and insulin (about 0.3 pg/cell) contents were comparable in the free and immobilized cells. In free RINm5F cells, the utilization of D-[5-^{3}H]glucose and oxidation of D-[U-^{14}C]glucose averaged, respectively, 65.2 ± 1.0 and 6.1 ± 0.2 pmol/10^3 cells per 120 min ($n = 9$ in both cases), yielding a paired oxidation/utilization ratio of 9.5 ± 0.2 %. In the cells immobilized on the two-dimensional supports, the paired ratio between D-[U-^{14}C]glucose oxidation and utilization was increased ($P < 0.001$) to 13.5 ± 0.4 % ($n = 10$). **Conclusions:** The present findings indicate that it is possible to immobilize tumoral islet cells to two-dimensional microsupports. The metabolic behaviour of the immobilized cells appeared well preserved, with even a higher ratio between D-glucose oxidation and utilization than in free RINm5F cells. Further work is in progress to extend these observations to normal islet cells immobilized to a suitable two-dimensional microsupport.

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FEEDING NOD MICE WITH PIG SPLENOCYTES INDUCES ACTIVE AND TRANSFERABLE MECHANISM WHICH MODULATES THE CELLULAR XENOGENEIC REACTION AGAINST PIG CELLS.

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Aim, materials and methods: We previouly suggested that oral administration of pig cells to NOD mice may constitute a novel approach to modifying xenogeneic cellular response against pig islet cells, and may be conducive to active suppression. However, in this preliminary study, firstly, the effect of oral administration of pig cells was only evaluated by " primary " proliferation (i.e when splenocytes from fed mice were then confronted for the first time to pig cells in vitro). In the present study, we have thus studied "secondary" in vitro proliferation in response to pig spleen and islet cells, i.e. after both feeding and subsequent in vivo (i.p.) exposure to pig cells. Secondly, induction of active cellular mechanism by feeding pig cells was only hypothetical. We have thus transfered (i.v.) splenocytes from mice pre-fed pig spleen cells and evaluated, in recipient syngeneic mice, "primary" (after transfer) and "secondary" (after both transfer and subsequent in vivo (i.p.) exposure to pig cells) proliferations. **Results:** NOD mice pre-fed with pig spleen cells (n=60) displayed highly increased "primary" proliferation of splenocytes in response to both pig spleen or islet cells ($p<0.0001$). Mice fed with pig spleen cells and then exposed in vivo (i.p.) to pig spleen cells (n=8) displayed lower "secondary" splenocyte proliferation in response to pig spleen cells than did control mice ($p<0.05$). Compared to transfer (i.v.) with spleen cells from control mice fed PBS, transfer with splenocytes from mice fed pig spleen cells increased the intensity of "primary" in vitro proliferation of splenocytes from recipient mice in response to pig spleen cells ($p<0.01$) and islet cells ($p<0.02$). This effect on "primary" proliferation was not decreased when recipient mice were irradiated prior to transfer. Moreover, recipient mice (n=4) exposed in vivo (i.p.) to pig spleen cells after being transfered (i.v.) with splenocyte from donor mice fed with pig spleen cells displayed decreased ($p<0.04$) " secondary " in vitro proliferation in response to pig spleen cells. In all those mice, similar decrease in secondary proliferation was also observed against pig islet cells, although to a lesser extent than against pig spleen cells. **Conclusion:** oral administration of pig cells induces active transferable mechanism which modifies primary and secondary xenogeneic cellular reactions against pig cells.

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HOMA TEST IN DIABETIC PATIENTS WITH SIMULTANEOUS PANCREAS AND KIDNEY TRANSPLANTATION.

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Endocrine function evaluation is a key point in the follow-up of pancreas transplantation. OGTT is the usual test performed to evaluate the metabolic status in patients with pancreas and kidney transplantation (PKTx). The Homeostatic Model Assessment (HOMA), is a shorter and easier test, that does not require previous diet, and that also estimates β-Cell secretory capacity (HOMA B), as well as insulin sensitivity (HOMA S), by applying a mathematical model to basal insulin and glycemia levels. **Aims:** To evaluate the usefulness of HOMA vs OGTT to determine insulin secretion in PKTx patients. **Material and Methods:** 68 PKTx patients (age: 40.6±6.0 yr; diabetes duration: 27.4±6.1 yr ; BMI 23.5±4.2 Kg/m^2) were studied 3.3±2.7 yr after a simultaneous pancreas (venous derivation and urinary drainage) and kidney transplantation. The HOMA test was performed (collecting samples for serum glucose and insulin levels at -10, -5 and 0 min thereafter) followed by OGTT (glucose and insulin levels at 0, 60,90, 120 min). **Results:** Insulin secretion evaluated by HOMA B (258.7±83.4) showed a positive correlation with the area under curve of insulin (9639.7±4693.7 mU/min.) obtained by OGTT (r:0.65, p<0.005). 50/68 patients (73.5%) presented a normal OGTT, 11/68 (16.2%) were intolerant and 7/68 (10.3%) were diabetic. Diabetic patients had statistically significant lower levels of HOMA B than normal patients by OGTT (162.14±29.9 vs. 275.10±79.7). The best threshold value of HOMA B level to predict diabetes by the receiver operating characteristic curve (ROC) was 280 (sensitivity 82%, specificity 40%). **Conclusions:** HOMA is a quick, cheap and useful test to evaluate insulin secretion in patients with PKTx. However, the specificity for predicting glucose tolerance abnormalities is low.

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FASL DEFICIENCY DOES NOT PREVENT ACUTE REJECTION BUT DECREASES EOSINOPHILIC INFILTRATION AFTER CONCORDANT ISLET XENOTRANSPLANTATION IN MICE

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Aims: CD4 T cells mediate rejection of allografts either directly through the Fas/FasL cytotoxic pathway or indirectly via the secretion of Th1-type cytokines. However, xenograft rejection may involve a CD4 T-cell response of the Th2 type, associated with eosinophilic infiltration. The possible role of the Fas/FasL pathway in the rejection of concordant islet xenograft was now investigated in FasL deficient mice. **Methods:** WF rats were used as pancreatic islet donors. C57BL/6 (B6) wild-type or $B6^{gld/gld}$ (FasL-deficient) mice were made diabetic at 6-8 weeks of age by an intraperitoneal injection of streptozotocin. About 600 freshly hand-picked islets were transplanted into diabetic recipients, which resulted in a return to normoglycaemia within 1.3 ± 0.2 days (mean ± SEM). Rejection was diagnosed when nonfasting glycaemia (monitored each day) was above 11.1 mmol/l on two consecutive bleedings. The mice were nephrectomized at the time of rejection for histological examination of the graft using haematoxylin/eosin staining. **Results:** All (n = 5) FasL-deficient mice rejected islet xenografts, with kinetics similar to that of wild-type mice : time to rejection was 14.4 ± 3.2 days in FasL-deficient mice *vs* 10.2 ± 1.2 days in wild-type mice (P > 0.2). Histological examination of grafts revealed destruction of most islets with an intense leucocyte infiltration. Graft infiltrates in wild-type mice consisted of lymphocytes together with eosinophils, whereas in FasL-deficient mice only few eosinophils were present (41 ± 8 *vs* 11 ± 5/0.0025 mm^2; P < 0.02). **Conclusions:** In this model of concordant xenotransplantation, the Fas/FasL pathway is not required for the induction of acute rejection but is critically involved in the intragraft recruitment of eosinophils.

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EXTRACELLULAR MATRICES FOR SUPPORTING LARGE MAMMAL PANCREATIC ISLET CELLS*.

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Aims: To investigate the potential of different extracellular matrices as support for large mammal islets, to be used in xenotransplantation studies. Materials and Methods: Large (LB, approximately 2 mm diameter) and small (SB, approximately 0.7 mm diameter) sodium alginate beads containing intact bovine islets (BI, prepared by collagenase digestion and density gradient purification) or dispersed bovine islet cells (DBIC, prepared by trypsin digestion of isolated islets) were prepared by suspending BI or DBIC in 2-3% sodium alginate, and then by converting the suspension into droplets by means of an air-driven droplet generator. CulthiSpher-S microcarriers (MC, by Percell Biolytica), a gelatin-based support, were generated by re-hydration of the native compound, and then cultured with DBIC at 37°C, to allow these latter to enter the microcarriers. Cell survival was assessed up to 1 month by hematoxylin-eosin (HE) and/or MTT staining, and by insulin secretion (IS) studies. Results: After 1 month from the preparation, cell survival by HE and/or MTT was: 22±3% with BI in LB, 35±7% with DBIC in LB, 81±8% with BI in SB, and 88±4% with DBIC in MC. Throughout the study period, and in comparison with that of free and intact bovine islets, IS from BI and DBIC in LB was reduced to about 1/5, IS from BI in SB was reduced to about 2/3, and IS from DBIC in MC was reduced to about 1/2. Sensitivity to glucose (IS at 16.7 mM glucose at least two-fold higher in comparison with IS at 3.3 mM glucose) was well maintained with BI in SB and DBIC in MC; sensitivity to arginine (IR at 3.3 mM glucose plus 20 mM arginine at least two-fold higher in comparison with 3.3 mM glucose) was well maintained with BI in SB. Conclusions: Small sodium alginate beads (that may provide protection against immunological reactions) are a suitable support for bovine islets; the use of dispersed islet cells within gelatin microcarriers could be of potential usefulness to minimize the well known phenomenon of insufficient supply of oxigen and nutrients to the central core of intact islets upon culture or transplantation. **Supported by the European Community, project "Bioartifical Pancreas", contract BMH4-CT98-9516*

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COMPARISON OF ACCEPTANCE OF PIG XENOGRAFTS IN THE GENERAL POPULATION AND PATIENTS WITH TYPE 1 DIABETES.

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Aim. The possibility of pig xenografts raises the question of their acceptance by society. No study of this type has been published in Europe, and no effort has been made to compare acceptance by the general population with that of patients potentially concerned by xenografts. **Material and methods.** We developed a survey by oral multiple-choice questionnaire. Our objectives were to quantify acceptance of xenografts, particularly from the pig, in a sample of the French population (n=800) and to determine whether it differed for 377 Type 1 diabetic patients. **Results.** The percentage of diabetic patients willing to accept a xenograft was significantly higher to that of the general population (63.7% vs 53.7%, p<0.01). The percentage of undecided individuals was similar among the two populations (10.8% vs 9.6%). Furthermore, among the general population, man, the monkey, the pig, the dog, and the cow were rated in order as preferred donor species, whereas among diabetic patients the pig was rated second after man (the mean rank of the pig was thus significantly higher for diabetic patients: 2.95 ± 0.11 vs 2.42 ± 0.07, p<0.01). No correlation was found between the duration of diabetes or the presence of secondary complications and the acceptance of a xenograft in general or from a pig in particular. Among the reasons for reluctance to accept a xenograft, sanitary risks relating to the xenograft were considered as frequently by diabetic patients and the general population (29.7 vs 23.5 %) and not more frequently from sanitary risks relative to any graft. The two populations indicated that additional information might ultimately change their opinions, but that a guarantee by medical authorities would not be sufficient to convince them. **Conclusion.** The possibility of xenografts is rather well accepted by the French general population in 1999. The number of undecided individuals suggests a need for better information. The acceptance of xenografts in general and pig tissues in particular is higher in Type 1 diabetic patients. The decision to use xenografts cannot be based simply on the expectations of patients, as individuals were less aware than the scientific community of the sanitary risks specifically relating to the xenograft.

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STIMULATED URINARY AMYLASE EXCRETION CORRELATES WITH ENDOCRINE PANCREATIC GRAFT FUNCTION

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Aims: In patients after combined pancreas and kidney transplantation (PKTx) with pancreatic duct drainage into the urinary bladder, a single examination of basal urinary amylase excretion rate (UAmER) will not indicate endocrine graft dysfunction. Our study was designed to establish whether UAmER following stimulation by food correlates with pancreatic graft insulin secretion. **Methods:** We examined six patients with Type-1 diabetes mellitus (4 women, 2 men, age range 29-52 yrs) after PKTx. At the time of examination, all patients were normoglycemic, with good renal graft function (mean serum creatinine 103.47±17.68 µmol/L). The pancreatic graft response to a food stimulus was monitored at a 3-hr interval after standard breakfast (55% sacharides, 30% fat, 15% protein). Basal parameters were assessed at a 1-hr interval before administration. Samples of urine (after catheterization) and blood to determine UAmER, serum free insulin (FIRI), and glycemia were collected at a 30-min interval. **Results:** Basal UAmER was 30.26±17.55 µkat/30min, basal FIRI 18.8±6.7 mU/L. Stimulation by food was followed by a rise in UAmER to a 1.45 to 6.02-fold of basal values with a peak between minutes 30 and 60 (66.16±55.25 µkat/30 min). FIRI levels rose to a 2.17 to 9.53-fold of basal values with a peak at minute 60 (82.9±91.9 mU/L). We demonstrated a significant correlation ($R=0.81$, $p<0.05$) between the stimulation indexes (ratios of peak stimulated to basal levels) of UAmER and FIRI. A patient with the lowest stimulation indexes developed hyperglycemia two days after the examination and pancreatic graft rejection was demonstrated (grade II by histology). **Conclusions:** Our preliminary results suggest that food-stimulated UAmER reflects well endocrine graft function and could be used as a non-invasive marker for detecting pancreatic graft dysfunction.

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PROGNOSTIC VALUE OF EARLY GRAFT FUNCTION AFTER PANCREAS TRANSPLANTATION ON LONGTERM GRAFT SURVIVAL

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Aims: Monitoring of pancreas graft function as far as the diagnosis of acute or chronic rejection is difficult. The aim of this study was to search for diagnostic parameters with prognostic value for longterm graft survival. **Methods:** 48 pancreas graft recipients, who could be followed for at least five years after successful transplantation were investigated. 17 graft recipients had a loss of pancreas graft function after five years (35%), whereas 31 recipients had an intact graft function with insulin-independence and normal serumglucose and HbA1c levels after 5 years. Diagnostic parameters like fasting blood glucose, HbA1c and results of an OGTT three months after transplantation, were compared between those who lost their graft and those with functioning graft. Groups were compared by Mann-Whitney-U-test or Fisher's exact test was applied for categorical variables. **Results:** No differences concerning HbA1c and fasting blood glucose were found between the groups. The measurement of insulin secretion did not reveal significant differences between the groups. Graft recipients with later graft loss displayed three months after transplantation more often an abnormal OGTT, however not significantly, than recipients with intact graft function five years after transplantation (70% vs. 43%, p=0,06). **Conclusions:** Investigations of glucose metabolism after pancreas transplantation serve as an indicator for present graft function, respictevely secretory capacity of pancreas graft. However, neither routine parameters like glucose and HbA1c levels nor glucose disposal and glucose-induced insulin release, early after transplantation, have prognostic value for longterm graft survival.

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Hypoglycaemia

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CONTRIBUTION OF ADRENALINE TO HYPOGLYCEMIA RESPONSES IN HUMANS.

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To establish the specific contribution of plasma adrenaline (Adr) to hypoglycemia (H) responses, 6 bilaterally adrenalectomized patients (BA-pt, 3M, 3F, age 43±3 yrs, BMI 26±1 kg/h^2) (mean ± SEM) were studied during stepped H (plateau plasma glucose, PG, of 90, 78, 66, 54, 42 mg/dl, hyperinsulinemic-hypoglycemic glucose clamp) and i.v. cortisol replacement to mimic plasma cortisol responses to H of 12 normal control volunteers (N). During H, Adr was barely detectable in BA-pt (0.03±0.01 nmol/l) vs N (5.02±0.39 nmol/l), whereas noradrenaline was greater (2.62±0.56 vs 1.91±0.22 nmol/l), but glucagon lower in BA-pt vs N (27±7 vs 55±10, pg/ml, $p<0.05$). Glycemic thresholds were lower (60±1 vs 55±1 mg/dl) and maximal responses of neuroglycopenic symptoms (S) (10.8±1.7 vs 6.8±0.9) greater in Ba-pt vs N ($p<0.05$). Total autonomic S (13.8±2.5 vs 15.6±2, p=NS) were similar between BA-pt and N, despite lower adrenergic S (heart pounding, tremor, anxiety, 3.4±1.3 vs 7.7±1.4), because cholinergic S (sweating, hunger, paresthesias) were greater (10.4±1.5 vs 7.9±0.7) ($p<0.05$). Cognitive function (CF, sum of z scores of 12 psichometric tests) deteriorated more in BA-pt vs N (-33.5±4.4 vs 24.4±1.9, $p<0.05$). When Adr was infused to BA-pt to mimic Adr responses of N, glucagon and noradrenaline responses, cholinergic and adrenergic autonomic S normalized (p=NS vs N), but CF remained more deteriorated than N ($p<0.05$). Conclusions: chronic deficiency of Adr: 1)blunts responses of glucagon to H. This raises the question of a permissive role of Adr in glucagon responses to H, 2)blunts adrenergic, but exaggerates cholinergic autonomic S, ultimately resulting in normal total S score. 3)The greater impairment in CF during H in BA-pt is likely to chronic cortisol, rather than Adr deficiency.

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IMPAIRED MEMORY FUNCTION FOLLOWING FREQUENT LONGSTANDING HYPOGLYCEMIA

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The increased risk of hypoglycemia in patients under intensified insulin therapy is well documented. One consequence is a reduction in hypoglycemia awareness, a potentially reversible situation. Little is known on the long-term effects of frequently recurrent episodes of hypoglycemia on cognitive function, esp. memory performance. **Aims:** To study the occurence and grade of cognitive, esp. memory dysfunction in patients with recurrent longstanding severe hypoglycemia (blood glucose less than 40 mg/dl at least once per week) due to intensified insulin therapy. **Materials and Methods:** 9 patients suffering from type I diabetes aged 24 to 63 years underwent neurological and comprehensive neuropsychological examination including the WAIS-R, the Word-Figure-Memory-Test, the Recurring Figure Test, the Complex Figure Test, Luria's list of words, the cancelling d test and the PSE-Test and cranial MRI. 9 healthy volunteers, mached with respect to age and education, served as a control group. **Results:** The two groups revealed similar results in the WAIS-R subtests. None of the patients presented with a pathological WAIS result, neither in the subtests nor with respect to the total score. In contrast , the patients did significantly worse than the controls in the memory tests ($p<0.05$). 6 of the 9 patients revealed pathological results in at least one of the memory tests performed. Cerebral MRI was normal in all patients. **Conclusions:** The results suggest memory disturbances as a complication of repeated severe hypoglycemia following intensified insulin therapy.

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ALCOHOL AND GLUCOSE COUNTERREGULATION DURING ACUTE INSULIN-INDUCED HYPOGLYCAEMIA IN TYPE 2 DIABETES.

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Background: In type 1 diabetes the risk of hypoglycaemia is increased by alcohol, which reduces gluconeogenesis, especially when accompanied by depleted glycogen stores. We have previously shown that alcohol does not acutely affect the glucose level despite an alcohol-induced rise in insulin levels. Furthermore, that the combination of alcohol and exercise does not elicit acute hypoglycaemia in type 2 diabetes. In type 1 diabetes alcohol impairs glucose counterregulation and recovery during insulin-induced hypoglycaemia. It is, however, unknown whether recovery from insulin-induced hypoglycaemia in type 2 diabetes is also compromised by alcohol. **Aim:** To assess the influence of alcohol on glucose counterregulation during acute insulin-induced hypoglycaemia in type 2 diabetes. **Protocol:** Eight type 2 diabetic subjects were examined twice following an overnight fast. A graded hyperinsulinaemic (1mU/kg/min) hypoglycaemic clamp was performed with infusion of 3-^{3}H-glucose in a primed (17 μCi) continous (0.17 μCi/min) manner, and a variable (200 μCi/l 20% glucose) manner to calculate glucose turnover. After a euglycaemic baseline (150-180 min) 200 ml water was taken either alone or with alcohol 0.4 g/kg. Hypoglycaemia (plasma glucose nadir 2,8 mmol/l) was then induced and the recovery period followed after discontinuation of insulin and variable glucose infusions. **Results:** On both study days the insulin levels, glucose levels, glucose nadirs and glucose kinetics were similar. Alcohol increased plasma lactate (AUC, recovery period) (244±30 vs 12±4 mmol/l x 240 min; p=0.00009) and decreased plasma FFA (AUC, recovery period) (95±13 vs 161±18 mmol/l x 240 min; p=0.0008). No differences were found in serum cortisol and growth hormone. Interestingly, peak glucagon was significantly higher without alcohol (200±17 vs 155±12 pg/ml; p=0.038).**Conclusion:** In contrast to type 1 diabetic subjects, type 2 diabetic subjects did not experience a deficient glucose recovery after alcohol. **Statistics:** ANOVA, and paired Student´s t test.

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INFLUENCE OF CAFFEINE ON NEUROPHYSIOLOGICAL FUNCTION DURING HYPOGLYCAEMIA

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Aims Caffeine is known to augment the sympathoadrenal response to hypoglycaemia and act as a psychostimulant. Its effects on nerouphysiolocial function during hypoglycaemia has not previously been studied.

Materials and Methods 16 healthy caffeine consumers (8 male, aged 24-36 years) were studied on 3 separate occasions. Using a hyperinsulinaemic clamp blood glucose was maintained at 4.5mmol/l for 90 min. Thereafter glucose was either (a) lowered to 2.5mmol/l for 90 min and subjects ingested 200mg caffeine, or (b) as for (a) but with ingestion of matched placebo. In a third study (c) euglycaemia was maintained throughout with 200mg caffeine given at +90 min. A test battery was administered during at each stage. This included 2 visual information processing tests measuring the ability to detect visual change (VCD) and visual movement (VMD). Full-field 16° pattern reversal visual evoked potentials (VEPs) were recorded.

Results The latency of the P100 component of the VEP increased significantly during hypoglycaemia ((a)5.69±1.54ms (b)2.57±1.29ms (c)-1.90±0.66ms; p<0.001) but there was no specific effects of caffeine. Considerable inter-subject variability in P100 latency was noted, thus subjects were divided into 2 groups by a median split of P100 latency increase(gp1:0.5ms, gp2:5.6ms). Subsequent analyses showed a group by condition interaction for P100 latency between the 2 conditions (gp1 5.88±2.28ms caff; 0.94±1.30ms placebo vs. gp2 5.50±2.22 ms caff; 4.43±2.23ms placebo, p<0.05). Changes in VCD and VMD during the clamp showed no significant difference between the conditions but the subjects who showed the largest P100 increase were also those who showed the most significant deterioration in performance of VMD (p<0.05)

Conclusions These data suggest that some individuals are more susceptible to changes in visual sensation as a result of hypoglycaemia and that caffeine exaggerates this independently.

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FREQUENCY AND PREDICTOR FACTORS OF NOCTURNAL HYPOGLYCAEMIA IN YOUNG PATIENTS WITH IDDM.

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Hypoglycaemia is the most common complication of insulin therapy. The frequency of hypoglycaemic episodes in young people has been reported between 7 and 44%, with the majority of episodes occurring night-time. ***Aim***: to evaluate the risk of nocturnal hypoglycaemia and to identify predictor factors in children and adolescence with IDDM. ***Materials and methods:*** we evaluated 60 children (28 female, 32 male), treated by 3/4 ii/day, admitted to our Department, mean age 16.0±4.5 yrs, duration of diabetes > 1 yrs, mean HbA1c=9.3±2.1. We performed a crossover study in the same patient during two consecutive nights using NPH with the same dose before and after supper respectively. Blood glucose (BG) was analysed at the hours 23, 2. and 7. Statistical analysis have been performed by non parametricmethods. ***Results***: no severe hypoglycaemia was observed, while the frequency of night time mild hypoglycaemia (BG<60 mg%) was 15/120 (12%) with no difference related to the time of NPH administration. The mean BG at 23 and at 2 with 3 injections/day was significantly lower compared to 4 injections/day with the same insulin dose ($p < 0.05$). Significant relations were found between BG at bed-time and at 2 am with both insulin regimen ($p<0.05$). ***Conclusions:*** High frequency of nocturnal hypoglycaemia is present irrespective of the time of NPH administration. Switch to 4 injections/day allows lower reduction of nocturnal BG with no difference in BG at awakening. Blood glucose at bed time predicts nocturnal hypoglycaemia.

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PRECISION OF RAPID BLOOD GLUCOSE DETERMINATION IN EMERGENCY MEDICINE

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Aims: In emergency medicine, blood glucose often has to be determined under extreme conditions in which the stressfulness of the situation, the need to act swiftly and the prevailing weather conditions create potential sources of error. **Methods:** In a prospective study of emergency callouts by fast-response doctors we investigated the precision of the reflectometric blood glucose determination under emergency conditions. 529 patients (age 62.4 ± 21 years, 56% male; 46 severe hypoglycaemias) to whom the emergency doctor was called were routinely fitted on-site with a peripheral venous indwelling catheter. Venous whole blood from the mandrin of the catheter was applied to the test strip and a glucose reading taken with the Glucotouch™ reflectometer. Immediately thereafter, blood from the catheter was collected in an EDTA tube for later formal blood glucose determination (glucose oxidase method), which occurred within 20 to 40 minutes. **Results:** The emergency glucose readings carried out at the scene of the emergency (mean 7.3 ± 4.4 mmol/l; range 0.61 – 27.7 mmol/l) correlated well with the formal readings obtained with the laboratory reference method (Pearson r = 0.98; linear regression analysis: slope 1.0 and axis intercept 1.74). Error-Grid-Analysis also showed good correlation of results: range A 96.7%, -B 2.5%, -C and -D 0.8%. With the method according to Bland et Altman, the mean of the differences was 0.144 mmol/l; 2 SD 1.83 mmol/l; minimum –7.05 mmol/l; maximum 4.4 mmol/l. **Conclusions:** The precision of venous blood glucose determinations performed by teams of emergency-response doctors working a daily changing rota was high. In only 0.8 % were there discrepancies which might have led to clinically relevant wrong decisions. The method is simple and not dependent on the haemodynamic status of the patients.

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METABOLIC AND BLOOD PRESSURE CONTROL IN UK ASIANS WITH TYPE 2 DIABETES AND CORONARY DISEASE

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Aims: To assess the extent to which glycaemic and blood pressure control in Asian patients with Type 2 diabetes undergoing coronary angiography conforms to standards set by the UKPDS, and to assess the extent to which reduction of cholesterol levels compares to standards set by the 4S and CARE studies. **Materials and Methods:** 58 Asian patients with type 2 diabetes undergoing coronary angiography in 4 centres in West Yorkshire were assessed. Standards for glycaemic control and blood pressure targets were extrapolated from the UKPDS and subsequent BDA guidelines, and total cholesterol levels from the 4S and CARE studies. **Results**: The mean fasting glucose was 9.3 mmol/l (95% CI 8.1-10.4), with 67% of patients having a fasting glucose level greater than 7mmol/l. The mean HbA1c of the group was 8.0% (95% CI 7.6-8.4). 71% of patients had an HbA1c higher than the target of 7%. The mean systolic bp of the group was 139mmHg (95% CI 133-145), and 53% of patients had a level >140mmHg. The mean diastolic bp was 79mmHg (95% CI 76-83), with 41% of patients having a level above 80mmHg. 48% of patients had a total cholesterol concentration of greater than 5.2mmol/l, and 64% greater than 4.8mmol/l, with the mean cholesterol being 5.4mmol/l **Conclusion:** The UKPDS, 4S and CARE studies have set new metabolic and blood pressure targets for patients with type 2 diabetes and suspected ischaemic heart disease. The population studied is at high risk of coronary artery disease and it should be expected that their control matches the targets set by these studies. These data demonstrate that this is currently not the case and greater efforts are needed to improve the outlook for this patient group.

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GLYCAEMIC AND BLOOD PRESSURE CONTROLS IN A COHORT OF 318 PATIENTS WITH TYPE 2 DIABETES.

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Aims: The aim of the study was to evaluate current anti-hyperglycaemic and anti-hypertensive treatment schemes as well as quality of metabolic and blood pressure control in a cohort of type 2 diabetic subjects, in view of the *United Kingdom Prospective Diabetes Study* (UKPDS) data. **Subjects and Methods:** 318 patients (196M; 122F) examined consecutively at the outpatient clinic were included. Age and known duration of diabetes were 63±11 and 13±9 years respectively (mean±1SD). Body mass index was 29.6±6.0 kg/m^2. **Results:** 9% of patients were on diet alone while 44% were treated with metformin and/or a sulfonylurea. 26% of subjects received insulin monotherapy and 19% had insulin combined with oral hypoglycaemic drug(s). 18 and 69% of patients had one or two daily insulin injections respectively (mean dose: 0.51±0.36 U/kg). Random plasma glucose was 178±28 mg/dl and HbA_{1c} 8.21±1.64%. (Un)treated hypertension was present in 59% of patients. Anti-hypertensive drugs included ACE-inhibitors (40%), calcium channel antagonists (20%), diuretics (20%) and β-blockers (18%), administered alone or in combination. Systolic and diastolic blood pressure were 147±22 and 86±12 mm/Hg. Neuropathy, retinopathy, microalbuminuria and macroangiopathy were present in 43, 33, 21 and 33% of patients respectively. When subjects were divided according to tertiles of HbA_{1c}, a higher prevalence of neuropathy and microangiopathy was found when HbA_{1c} was above 8% (tertiles 2 and 3) ($p<0.02$). This was also observed for hypertension ($p<0.002$) and macroangiopathy ($p<0.05$). **Conclusions:** In a type 2 diabetic population followed at a University Center, overall glycaemic control remains above the satisfactory levels inferred from the UKPDS data. In contrast, blood pressure control was deemed adequate.

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GLYCEMIC IMPROVEMENT IN TYPE 2 PATIENTS USING STAGED DIABETES MANAGEMENT.

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Staged Diabetes Management™ (SDM) is a program of clinical practice guidelines designed to improve glycemic levels within specific time periods. Its use is targeted and tailored to primary care physicians (PCPs), non-physician diabetes case managers and diabetes educators. **Aims:** We evaluated the impact of SDM on the health status of 131 predominantly Type 2 diabetes patients with sub-optimal glycemic control (HbA1c>7.4%) during a one year study period and one year follow-up. **Materials & Methods:** PCPs and case managers trained in SDM co-managed study patients. Patient allocation to case managers was based on HbA1c level, presence of co-morbidities and care-giver type/training. Clinical and resource utilization data were collected prospectively during the study year. A 12-month, non-SDM, pre-study period served as the control. **Results:** The mean 12 month pre-study and baseline HbA1c levels were 9.7% and 9.6% respectively. After 12 months of SDM intervention, the mean HbA1c level declined to 8.5% (Δ=-0.99%; p<0.001) from baseline values. Improvements were observed (p<0.05) in the performance of patient self-management activities (blood glucose monitoring, foot exams, dietary practice) and patient satisfaction of diabetes care. As glycemic control improved and SDM became the standard of care during the study year, resource utilization measured by clinic and telephone consultations decreased. The mean number of clinic visits per patient declined from 2.6 in the first 3 months of the study to 1.8 during the last 3 months. Similarly telephone consults declined from a mean of 2.6 to 1.4 during the same time frame. **Conclusion:** SDM is effective in achieving improvements in glycemic control, diabetes knowledge, patient self-management, and satisfaction with care of Type 2 diabetes patients. Resource utilization decreased with improvement in glycemic control during the study year.

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INCIDENCE AND OUTCOME OF ASYMPTOMATIC BACTERIURIA IN NIDDM FEMALES OVER A TWO YEAR FOLLOW-UP PERIOD AND ASSOCIATION WITH RISK FACTORS.

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Aims: Aim of our study was estimating the incidence of asymptomatic bacteriuria (AB), in NIDDM females, associate it with any relevant parameters and access treatment outcome over a 2 year period.

Patients-Methods: We prospectively studied over a 2 year period (m.follow-up: 91.8±30.4 weeks) 154 patients (m.age 61.3±10.5 yrs , m.duration of Diabetes [D] 10.8 yrs) followed in our D Clinic and screened them for AB, associated the results with their demographic, history, complications and glucaemic control data, as well as antibiotic (cotrimoxazole or ciprofloxacin) 14d regimen outcome. Analysis was done via an IBM compatible computer using the EPI5-INFO (CDC/WHO) 1993 program. Statistical analysis was performed with t- and Yates corrected chi square test.

Results: We found AB in 39 of 154 patients (cumulative incidence 25.3%) with 17 positive on baseline and another 22 during follow-up, with *E.coli* (75%), *Proteus* (9.5) and *Klebsiella* (6%) been the most frequent isolates. There was no difference found in age, D duration, kind of D treatment, renal function or glycaemic control, as expressed by HbA_{1-c}, throughout follow-up, between AB+ve and no AB patients. The presence of D microangiopathy complications (nephropathy and/or neuropathy) was associated with AB, (23.1% v 12.2%, p=0.09) but not significantly. Most strongly associated with AB was history of previous urinary tract infections, (71.4% v 24.8%, p<0.001). The outcome of antibiotic treatment, defined as eradication at 6 month follow-up, was 52.2% in treated AB episodes, while 50% of untreated, after informed consent, resolved at the same follow-up period.

Conclusuion: AB was found in a considerable percentage (25.3%) of NIDDM females, in a two-year prospective follow-up. The major risk factor for AB was history of previous urinary tract infection. Antibiotic treating of AB was not proven greatly beneficial in the long-term outcome.

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INSOMNIA IS MORE PREVALENT IN DIABETIC THAN NON-DIABETIC SUBJECTS AND PREDICTS MORTALITY

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Sleep disturbances (SP), e.g. insomnia and early awakening, are common health problems to many middle-aged and elderly people. Previous studies have reported on a higher prevalence of SP in subjects with type-2 diabetes (DM2) compared to non-diabetic controls. It is still unclear whether this finding is confounded by differences in age, sex, or obesity, and whether SP similarly predicts mortality in subjects with or without (DM2). **Aims:** To investigate SP by a self-administered questionnaire, used in a population-based sample of middle-aged men (577 with known DM2 or fasting B-glucose >6.7 mmol/L, 21.867 non-diabetics), and women (253 DM2, 10.649 non-diabetics) in the city of Malmö, Sweden. **Methods:** Questions were asked about difficulties in falling asleep, early awakening, and a regular intake of hypnotic drugs. Prevalence rates were adjusted for age and body mass index (BMI). Register-based follow-up for total mortality was carried out for a total of 18 years. **Results:** Females with diabetes were significantly more frequently having *"Difficulties in falling asleep"* than non-diabetic females, adjusted risk ratio (RR) 1.54 (95%CI 1.17-2.02), as well as *"Problems with early awakening"*, RR 1.42 (1.09-1.86). The consumption of hypnotic drugs more than three times a week was therefore increased, RR 1.99 (1.22-3.23). Corresponding adjusted risk ratios for diabetic males were non-significant, with RR 1.06 (0.82-1.37), RR 1.24 (0.97-1.58), and RR 1.37 (0.85-2.21). Total mortality was increased in all subjects with a combination of sleep problems, adjusted for traditional risk factors, and was regardless of diabetes status. **Conclusions:** Sleep problems are common in diabetes patients, especially females, compared to non-diabetics. This is not confounded by differences in age or obesity. Sleep problems can predict total mortality in both diabetes patients and non-diabetics.

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RELATIONSHIP BETWEEN IRON STATUS AND DIABETES IN ANTI-HEPATITIS C VIRUS POSITIVE PATIENTS: A CASE-CONTROL STUDY.

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Several reports have shown an association between Hepatitis C Virus (HCV) infection and diabetes. On the other hand, it is well known that HCV infection is characterized by increased iron storage. Recently it has been demonstrated an association between iron stores and the incidence of diabetes. **Aim:** To evaluate whether anti-HCV positive diabetic patients have higher iron deposits in comparison with HCV positive non diabetic patients. **Material and Methods:** We study 123 anti-VHC positive subjects: 55 diabetic and 68 non-diabetic patients, matched by age and gender. The patients were classified in two groups according to liver biopsy findings : chronical hepatitis or cirrhosis. Iron stores were stimated by determination of serum ferritin (immunoassay). Any patients presenting overt acute or chronic hemolysis or haemorrhage were excluded from the study. Statistics: χ^2, t-Student. Serum ferritin data were log-transformed in view of their skewed distibution. **Results:** Serum ferritin levels were higher in anti-HCV diabetic patients in comparison with anti-HCV non diabetic patients (302.4 ± SEM 50.5 vs. 80.3 ± SEM 11.4 ng/ml; p<0.0001). The difference remained at significant level when we consider the histological degree: chronic hepatitis (301.4 ± SEM 88.4 vs. 80.7 ± SEM 99.4 ng/ml; p=0.03) and cirrhosis (282.2 ± SEM 83.3 vs. 83 ± SEM 68.1 ng/ml; p=0.05). **Conclusions:** Our results cleary demonstrate that anti-HCV positive diabetic patients have higher iron stores than anti-HCV positive non diabetic patients. In addition, these data suggest that iron stores in VHC infected patients play a role in the increased risk for the development of diabetes.

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HEPATIC IRON CONCENTRATIONS IN DIABETIC AND NON DIABETIC PATIENTS WITH HAEMOCHROMATOSIS.

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Measurement of hepatic iron using quantitative chemical analysis of liver biopsies contributes to the diagnosis of haemochromatosis (H^+) and is of value in assessing the extent of iron stores. **Aims:** To compare hepatic iron concentration (HIC) and hepatic iron index (HII) in (1) patients with and without H^+ and in (2) haemochromatosic subjects with and without diabetes. **Patients and Methods:** HIC, determined by atomic absorption spectrophotometry, and HII (HICx[55.8xage(years)]$^{-1}$) were measured in a group of 23 patients with H^+ and in 15 controls suffering from various non-haemochromatosic liver diseases. Diabetes was present in 8 patients with H^+ (H^+Diab^+; age: 58 years; age at diagnosis of H^+: 55 years; median). In 15 H^+Diab^- subjects, age was 54 and age at H^+ diagnosis 53 years (*NS vs.* H^+Diab^+). **Results:** Plasma ferritin in patients with H^+ was 1367 µg.L^{-1} (*vs.* 333 µg.L^{-1} in non haemochromatosic subjects, p=0.001). HIC in patients with and without H^+ were 7000 and 375 µg/g dry liver (p<0.001). Corresponding HII were 2.8 and 0.14 (p<0.001), respectively. Plasma ferritin was not significantly different in H^+Diab^+ and H^+Diab^- patients. HIC and HII in H^+Diab^+ subjects were 6950 µg/g and 2.6 (*vs.* 8700 µg/g and 2.8 in H^+Diab^- patients; *NS*). Haemochromatosic patients with cirrhosis had higher iron stores than non cirrhotic subjects (HIC: 10.000 *vs.* 6800 µg/g; HII: 3.4 *vs.* 2.0). **Conclusions:** In H^+, HIC and HII are significantly higher than levels found in other non-haemochromatosic liver conditions. HIC and HII are comparable in H^+ subjects with or without diabetes. This indirectly suggests that in H^+, the extent of iron storage in hepatocytes is not associated with a higher risk for developing secondary diabetes.

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Lipid evaluation and glucose metabolism in HIV-infected patients prior and after initiation of protease inhibitor therapy.

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Background: Metabolic side effects (hyperglycaemia, insulin resistance, diabetes mellitus, dyslipidemia, lipodystrophy) have been attributed to the use of protease inhibitors (PI), but the suspected causative role of PI is still unproven. Many of these studies were cross-sectional and compared patients already taking PI with controls.
Methods: We measured Insulin resistance (IR), glycemia, insulinemia and lipid profile prior and after initiation of PI. IR was measured with the homeostasis model assessment (HOMA) using the fasting glucose and insulin concentrations. An oral glucose tolerance test (OGTT) was performed only after initiation of PI therapy. 106 male patients, treated for at least 12 months with PI, were enrolled in this study. HIV-viral load was also measured as a marker of efficacy and compliance to PI therapy.
Results:

	Prior PI therapy	After PI therapy	p
glycemia mmol/l	4.94+/- 0.68	4.86+/- 0.66	0.42
Log insulinemia	0.76+/- 0.26	0.78+/- 0.35	0.93
Log IR	0.1 +/- 0.29	0.11+/- 0.38	0.92
Cholesterol mmol/l	4.38+/- 0.99	5.46+/- 1.36	0.001
HDL chol. mmol/l	0.9 +/- 0.28	1.07+/- 0.29	0.001
Log trigly	0.17+/- 0.25	0.27+/- 0.23	0.001
BMI kg/m^2	22.3 +/- 2.68	23.7+/- 3.19	0.05

Triglycerides and cholesterol increased significantly with PI therapy. Fasting glycemia and insulinemia, and insulin sensitivity were not significantly modified after the PI therapy; however, 12.2% of patient had glucose intolerance (8.5%) or diabetes mellitus (3.7%) under treatment. PI therapy significantly increased BMI. We observed a strong negative correlation between HIV-viral load and the following parameters after PI therapy: BMI (r:- 0.31; p<0.01), waist circumference (r-0.28: ; p=0.05), fasting glycemia (r: -0.26 ; p<0.01), cholesterol (r:-0.25 ; p= 0.01). **Conclusions:** Our longitudinal study confirms that dyslipidemia is strongly associated with the use of PI. Dyslipidemia, weight gain and increased waist circumference seem to be associated with PI efficacy. By contrast to previous cross-sectional studies, PI therapy has only a minor impact on glucose metabolism and insulin sensitivity.

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INTENSIVE CONTROL OF BLOOD GLUCOSE IMPROVES THE METABOLIC SYNDROME IN PATIENTS WITH TYPE 2 DIABETES MELLITUS.

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Aims: To examine whether intensive glycaemic control improves clinical, biochemical and body composition parameters in patients with poorly controlled type 2 DM. **Methods:** 34 subjects, $HbA_{1c} \geq 9.0\%$, were randomised to intensive (IC) or usual (UC) glycaemic control for 20 weeks. Body composition assessed by DEXA. Subjects received insulin, metformin or sulphonylureas or combinations. Targets were IC $HbA_{1c} < 7\%$; UC avoidance of fasting blood glucose ≥ 17mmol/L.

Results: Table1 Patient characteristics at baseline and week 20

	Intensive control (n=15)		**Usual control** (n=19)	
Age (years)	57.1 (6.3) 7 female		55.1 (9.3) 9 female	
	baseline	**week 20**	**baseline**	**week 20**
% HbA_{1c}	10.9 (1.2)	7.9 (0.9)*†	10.3 (1)	10.4 (1.3)
Cholesterol	5.3 (0.9)	5.0 (0.8)	5.0 (0.8)	5.1 (1.0)
Triglyceride	1.7 (0.7)	1.5 (0.6)*	2.0 (1.2)	2.0 (1.0)
Weight (kg)	93.0 (15.5)	96.3 (16.2)*†	80.6 (12.9)	81.2 (13.2)
BP (mm Hg)	133/79	131/77	128/80	129/79

Values mean ± SD, *p≤ 0.04 baseline vs week 20; † p≤ 0.005 change in IC vs change in UC; all units in mmol/L unless stated. No change in LDL or HDL.

Table 2 DEXA body composition changes (Δ) at week 20

	Intensive control	**Usual control**
Fat mass Δ (kg)	+ 1.68 (2.44)*	+ 0.49 (2.13)
Trunk fat Δ (kg)	+ 0.90 (1.87)	+ 0.53 (1.68)
Fat free mass Δ (kg)	+ 1.75 (2.94)*†	+ 0.28 (1.63)

Values mean ± SD, *p≤ 0.04 Δ from baseline; † p=0.07 IC versus UC

Conclusion: While intensive glycaemic control increases body weight, fat free mass increases more than fat mass and is accompanied by favourable changes in lipids and no significant change in blood pressure.

PS 65

Oral Drugs: α Glucosidase Inhibitors

834

PERIPHERAL INSULIN SENSITIVITY AND LIPID METABOLISM IN OBESE RATS AFTER CHRONIC TREATMENT WITH *ACARBOSE*

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Aims: We have investigated the effect of chronic treatment with *acarbose* on fasting plasma glucose, insulin, triglyceride, cholesterol and NEFA concentrations, as well as on the glucose and insulin excursions during OGTT, in obese diabetic Wistar (WDF) rats. **Materials and Methods:** 45 mature (mean age = 4.2 ± 0.1 months) male WDF rats were randomly distributed to one of three treatment groups (no *acarbose*, 20 and 40 mg of *acarbose*/100g of chow respectively). After 3.5, 7.5 and 11.5 months, animals were tested for glucose tolerance by means of an OGTT, and their respective metabolic profiles were determined. Control determinations were done in obese and age-matched lean animals before the start of the trial. **Results:** Obese rats exhibit higher body weight and fasting blood glucose (183±12 mg/dl), insulin (529±175 μU/ml), triglyceride (221±7 mg/dl) and cholesterol (221±3 mg/dl) concentrations, as well as glucose intolerance compared to lean animals. In all groups, a decrease in plasma glucose throughout the study is observed. However, whereas in the group receiving no *acarbose* this is accounted for by an increase in plasma insulin (up to 880 ±100 μU/ml after 11.5 months), in rats treated with *acarbose* the reversion of the diabetic state takes place without increments in hormone concentration, suggesting an improvement of the insulin sensitivity. In regard to glucose tolerance, similar results were obtained. Thus, the ΔGlucose area during OGTT decreases in all three groups, but the ΔInsulin area increment is significantly higher in the animals fed without *acarbose*, indicating a higher insulin resistance in this group. Concerning lipid metabolite, rats treated with *acarbose* for 3.5 and 7.5 months show lower plasma triglyceride and NEFA concentrations, and the same is observed for cholesterol at the highest dosage of the drug. **Conclusions:** Chronic treatment with *acarbose* of WDF rats improves the glycemic and lipidic control as well as the glucose tolerance, with a lower demand of pancreatic insulin than in untreated rats. The data suggest that chronic modulation of glucose and insulin excursions after meals improves insulin sensitivity in WDF rats.**Acknowledgements:** This work was supported by Química Farmaceútica Bayer S.A. (Spain)

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EFFECTS OF ACARBOSE AND GLIBENCLAMIDE ON INSULIN SENSITIVITY AND SUBSTRATE OXIDATION IN TYPE 2 DIABETIC PATIENTS

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Glycogen and glucose oxidation are related with the degree of insulin resistance. The aim of this double blind study was to compare the effects of acarbose and glibenclamide on substrate oxidation and insulin sensitivity. 48 type 2 diabetic patients (mean age 59,6 years, mean BMI 27.0 kg/m^2) were randomized to 3 different treatment regimes: 100 mg tid acarbose (n=17) or 1 mg tid glibenclamide (n=16) or 1 tid placebo (n=15). Before and after the 16 weekly treatment period we measured insulin sensitivity by euglycemic hyperinsulinemic clamp technique and substrate oxidation by indirect calorimetry. The body weight decreased in the acarbose and placebo group, but increased in the glibenclamide group (acarbose: before 79.0 after 77.0 kg, glibenclamide: 78.6 vs 79.5 kg, placebo: 78.4 vs. 76.9 kg), the postprandial insulin levels showed the same changes. Glycemic control was more improved in the glibenclamide group than in the acarbose group, but worsened in the placebo group. Glycogen oxidation raised in the acarbose and glibenclamide treated patients, but decreased in the placebo group, glucose oxidation increased in the acarbose group, was unchanged in the glibenclamide group and decreased in the placebo group (glycogen oxidation: acarbose: before 155.9 after 192.3 g/d, glibenclamide: 132.2 vs 173.8 g/d, placebo: 148.6 vs 135.0 g/d; glucose oxidation: acarbose: 251.0 vs. 284.8 g/d, glibenclamide: 220.9 vs. 219.9 g/d, placebo: 263.9 vs 227.0 g/d). Insulin sensitivity increased in the acarbose and placebo group, but was unchanged in the glibenclamide group with significant differences between acarbose and glibenclamide as well as placebo and glibenclamide (Mc (mg*(kg body weight*insulin*min)$^{-1}$): acarbose: 2.25 vs 3.26, glibenclamide: 1.83 vs. 1.71, placebo: 2.75 vs 3.38, p=0,003). In conclusion glibenclamide was more effective in improvement of glycemic control and has increased glycogen oxidation, but insulin sensitivity was unchanged. Acarbose has raised insulin sensitivity by improved glycogen and glucose oxidation.

836

ACARBOSE IMPROVES GLYCAEMIC CONTROL IN PATIENTS WITH INSULIN - TREATED TYPE 2 DIABETES.

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Aims: The potential benefit of acarbose in improving glycaemic control in patients with stable insulin-treated Type 2 diabetes was studied.
Materials and Methods: The trial was multicentre, double-blind, parallel-group, and placebo-controlled. After a two week placebo run-in period, 154 patients were randomised to acarbose 100mg tds or a matching placebo for 26 weeks. There was a 6 week forced titration period. The dose of insulin was to be kept stable.
Results: Patient characteristics were as follows: male 58 %, median age 62 years (range 34 - 75), median duration of diabetes 10 years (range 2 - 35), mean BMI 28.5 ($\pm$ 4.4 SD), and mean baseline HbA_{1c} 8.1% (range 6.3 - 10.2). The baseline HbA_{1c} was similar in both treatment groups. Of the patients randomised, 36 (48%) in the acarbose group and 7 (9%) in the placebo group withdrew prematurely due to side-effects. After hypoglycaemia, the most common side-effects in the acarbose group were gastrointestinal, these side-effects are well recognised with acarbose and consistent with its mode of action. There were no statistically significant differences between treatment groups in hypoglycaemic episodes. At the end of treatment, mean HbA_{1c} level was 7.8% in the acarbose group and 8.4% in the placebo group. An intention-to-treat comparison of the two treatment groups, showed a mean decrease in HbA_{1c} of 0.3% in the acarbose group and a mean increase of 0.3% in the placebo group (p=0.001), with a difference in mean HbA_{1c} levels of 0.6%. There was no change in median insulin dosage in either treatment group throughout the study. There were no statistically significant, or clinically relevant differences in fasting plasma glucose between treatment groups.
Conclusions: This study shows that the use of acarbose in patients with insulin treated Type 2 diabetes can significantly improve glycaemic control.

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THE EFFICACY AND SAFETY OF ACARBOSE IN THE TREATMENT OF TYPE 2 DIABETES MELLITUS IN ELDERLY PATIENTS.

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The efficacy, safety and tolerability of acarbose was examined in elderly patients with type 2 diabetes in a multicenter, double-blind, placebo-controlled study. All patients were advised on a weight-maintaining diet and any oral hypoglycemic agent was discontinued. After a 4-week placebo run-in period, 192 patients were randomly assigned to receive acarbose ($n = 86$) or placebo ($n = 94$) for 12 months ; the study drug was titrated gradually up to 100 mg TID. The mean age was 70 years for both placebo and acarbose patients and the mean duration of diabetes was 4.8 and 5.8 years respectively. The mean baseline HbA_{1c} was 7.1% for the placebo group and 7.2% for the acarbose-treated patients. After 1 year of treatment, the HbA_{1c} increased by 0.31% in the placebo group and decreased by 0.27% in the acarbose group for a significant placebo-substracted mean acarbose effect of 0.58% reduction ($p < 0.0001$). While placebo patients had an increase of 0.4 mmol/L in mean fasting plasma glucose, the acarbose patients had a decrease of 0.3 mmol/L ($p = 0.0023$). Acarbose patients had significantly lower post-prandial plasma glucose rise compared to placebo patients (Δ AUC : 4.30 vs 6.54 mmol•h•L ; $p < 0.0001$). Post-prandial serum insulin peaks decreased to a greater extent in the acarbose-treated patients (-141.6 vs -81.3 pmol/L) but did not reach significance ($p = 0.11$). Five patients (5%) on placebo and 13 patients (14%) on acarbose discontinued prematurely from the study due to adverse events ; the most common adverse events in the acarbose group were gastrointestinal. These observations demonstrate that acarbose is effective in improving glycemic control in elderly patients with type 2 diabetes and that the drug is safe and well tolerated.

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MIGLITOL IN COMBINATION WITH METFORMIN IMPROVES GLUCAEMIC CONTROL IN TYPE 2 DIABETICS

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Aims: The majority of patients with type 2 diabetes receive oral hypoglycaemics such as metformin as well as dietary adjustment. We have performed a multicentre, randomized, double-blind, placebo-controlled, parallel group study to investigate the long-term efficacy and safety of the α-glucosidase inhibitor miglitol in patients insufficiently controlled with diet and metformin therapy. **Materials and Methods:** Adult diabetic patients taking metformin 1500-2550 mg/day were randomized to receive additional placebo (n=75) or miglitol (n=78) for at least 28 weeks. The miglitol dose was increased stepwise from 25 to 100 mg/day.

Results:	HbA_{1c} (%, baseline adjusted)	1h postprandial glucose (mg/dl, baseline adjusted)	Fasting plasma glucose (mg/dl, baseline adjusted)
Metformin + placebo	8.67	283.8	208.2
Metformin + miglitol	8.26	248.7	195.3
Difference	p=0.03	p=0.0007	p=0.15

When miglitol was added to metformin, significant decreases in HbA_{1c} (p=0.03) and 1h postprandial blood glucose (p=0.0007) in favour of miglitol were observed. A trend in favour of miglitol was also seen for the secondary efficacy parameters fasting triglycerides (TG), postprandial TG and fasting insulin. Adverse events (AEs), most of which were gastrointestinal, mild and not treatment-limiting, were reported by only 8% more patients in the metformin + miglitol group than in the metformin + placebo group; there were no significant changes in laboratory parameters or vital signs. 19 patients (11 on metformin + miglitol, 8 on metformin + placebo) withdrew prematurely due to AEs. **Conclusions:** In type 2 diabetics, combination of miglitol and metformin significantly improves glycaemic control compared to metformin monotherapy, mainly through reduced postprandial glucose levels. The combination therapy is safe and well tolerated.

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IMPROVED GLYCAEMIC CONTROL WITH MIGLITOL IN TYPE 2 DIABETICS INSUFFICIENTLY CONTROLLED ON ORAL HYPOGLYCAEMICS

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Aims: Many patients with severe type 2 diabetes do not achieve normoglycaemia even with both diet and oral hypoglycaemics. This multinational, double-blind, placebo-controlled study investigated the long-term efficacy and safety of the α-glucosidase inhibitor miglitol in patients insufficiently controlled (HbA1c ≥7.5% -≤10.5%) on sulfonylurea (SU) + metformin (MET) therapy. **Materials and Methods:** Adult diabetic patients receiving glibenclamide 7-20 mg/day + metformin 500-850 mg/day were randomized equally (n=77/group) to receive additional miglitol (MIG) or placebo for 24 weeks. The miglitol dose was increased stepwise from 25 to 100 mg/day.

Results:	HbA_{1c} (%, baseline adjusted)	1h postprandial glucose (mg/dl, baseline adjusted)	Fasting plasma glucose (mg/dl, baseline adjusted)
SU+MET+placebo	8.64	277.0	192.0
SU+MET+MIG	8.28	242.0	178.0
Difference	p=0.04	p=0.0009	p=0.10

Against background sulfonylurea and metformin, miglitol produced greater decreases in HbA_{1c} and postprandial glucose than placebo. Reduction in fasting blood glucose was greater on miglitol but the difference was not statistically significant due to high individual variation. There was a trend in favour of miglitol for fasting and postprandial triglyceride levels. Adverse events (AEs) were reported by only 10% more patients in the SU+MET+MIG group than in the SU+MET+placebo group; most were gastrointestinal, mild and not treatment-limiting, and there were no significant changes in laboratory parameters or vital signs. 7 patients withdrew prematurely due to AEs in the SU+MET+MIG group and 6 in the SU+MET+placebo group. **Conclusions:** Addition of miglitol to SU+MET significantly improves glycaemic control in type 2 diabetics, largely through reduced postprandial glucose levels, is well tolerated and avoids the need to increase the dosages of the background oral therapy.

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COMPARISON THE EFFECTS OF ACARBOSE ON LOW AND HIGH GLYCEMIC INDEX MEALS

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Aims: The aim of this study was to evaluate the effect of acarbose (100 mg single dose) on postprandial glucose rise after 50 g carbohydrates from Corn Flakes which has high glycaemic index (GI:123) and All Bran which has low glycaemic index (GI:72). **Materials and Methods:** 10 voluntary type 2 diabetics (mean chronologic age: 51.1±5.4 yr, mean diabetes duration: 3.7±2.4 yr, mean BMI: 27.2±3.1 kg/m^2, mean HbAıc level 8.5±0.7%) consumed low glycemic index meal (LGI, 110 g All Bran with 200 ml milk,) and high glycemic index meal (HGI-60 g Corn Flakes with 200 ml milk,) with and without acarbose one week intervals, Blood samples taken before and at 30th, 60th, 90th, 120th, 180th min. after consuming the test meals within 10 min. Glucose and insulin levels were analyzed and Incremental Area Under the Curve (IAUC) were obtained. **Results:** The postprandial blood glucose increase was significantly lower after the LGI than HGI ($p<0.02$). The addition of 100 mg acarbose to LGI meal significantly reduced IAUC for glucose (7125±2096 mg.min/dl, 5146 ± 2310 mg.min/dl, $p<0.02$). Beneficial effects of LGI meal were roughly close to the effects of acarbose when added to HGI meal (7125±2096 mg.min/dl., 6160±1926 mg.min/dl). **Conclusions:** Adding a unique dose acarbose to LGI meal was even much more effective in decreasing postprandial serum glucose than HGI meal. We conclude that even in highly compliant patients to meal planing with LGI foods, adding an α-glucosidase inhibitor to meals containing foods with LGI, may result in better response in avoiding high glycemic excursions.

PS 66

Oral Drugs: Metformin

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EFFECT OF 6 MONTHS OF INSULIN THERAPY, ACUTE HYPERINSULINEMIA AND ACUTE HYPERGLYCEMIA ON ENDOTHELIAL FUNCTION IN TYPE 2 DIABETES

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Aims. The UKPDS suggested that insulin therapy may reduce macrovascular events. We examined effects of insulin therapy, acute hyperglycemia and acute hyperinsulinemia on endothelial function in type 2 diabetes.

Materials and Methods. We determined *in vivo* endothelial function 1) before and during (at 6 mos) of metformin therapy (MET, n=6) and 2) before and during (at 6 mos) of metformin and bedtime intermediate acting insulin therapy (INS +MET, n=18). The acute effects of an insulin injection and of ambient glycemia were also determined by restudying 7 patients with greatest improvements in acetylcholine (ACh) resposes twice, after omission of their bedtime insulin injection (INS-INJ) and after matching glycemia to preinsulin therapy levels (GLUC). 26 matched normal subjects were studied as controls (CONT). *In vivo* endothelial function was determined from blood flow responses to intra-arterial infusions of endothelium-dependent (ACh 15 μg/min) and - independent [sodium nitroprusside (SNP) 10 μg/min] vasoactive agents.

Results.

Flow	INS + MET		MET		INS-INJ		GLUC
(ml/dl·min)	Before	During	Before	During	Yes	No	
ACh 15	7.5±0.7^X	10.8±1.6*	8.9±1.4^X	9.1±1.0^X	15.5^X±2.2	9.0±1.8*	10.9±2.1
SNP 10	11.0±0.8	13.0±0.7	10.8±2.0	10.5±2.7	13.6±1.0	12.2±1.0	14.0±1.7
fP-Glucose	12.7±0.6^X	7.3±0.3^X*	11.3±1.5^X	12.9±1.1^X	7.1±0.6^X	8.4±0.3^X	12.6±0.8^X

$^X p<0.05$ vs CONT, *$p<0.05$ before vs during and Yes vs No

The increase in blood flow during infusion of ACh correlated with the increment in insulin concentrations during insulin therapy ($r=0.49$, $p<0.05$).

Conclusions. We conclude that INS + MET improves both endothelium-dependent (due to acute hyperinsulinemia), and -independent (due to factors other than changes in insulinemia or glycemia) vasodilatation. These data support the idea that hyperinsulinemia induced by insulin therapy is beneficial rather than harmful.

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THE EFFECT OF METFORMIN ON SERUM B12 LEVELS : IS THERE ANY CLINICAL IMPORTANCE?

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Aim: Aim of this study was to evaluate the effect of metformin on several biochemical and haematological parameters and its possible clinical significance. Material and Methods: Twenty four type 2 overweight , newly diagnosed , diabetic subjects (10 males, 14 females, age 55±12 yrs), started treatment with metformin 850mg daily after a 3-month diet period. If glycemic control was not achieved after one month (HbA1c >7.5% and/or FBG >7.0 mmol/l) the dose of metformin increased to 850mg twice daily. Blood samples were collected at baseline and 3 months after the start of metformin. Paired t-test was used for statistical analysis. Results : Results are presented in the table

		Baseline	After 3 months	p
B12	pmol/l	689±274	370±160	0.023
Folic acid	ng/ml	8.12±6.7	9.2±8.4	NS
Cholesterol	mmol/l	6±0.8	5.4±0.7	0.02
HDL	mmol/l	49±14	51±12	NS
Triglycerides	mmol/l	2.1±0.8	1.6±0.5	0.003
Glucose	mmol/l	12.4±3.1	7.7±2	0.04
HbA1c	%	9.5±2.8	8.6±1.1	0.03
MCV	fl	90.3±2.6	91.5±2.5	0.001
Body Weight	Kg	96.4±23	93.6±22	0.009

Conclusions: There is a statistically significant decrement of the serum B_{12} levels, which is already present after a three-month treatment. The significant drop of B12 levels may indicate the need for regular serum B_{12} measurements at least in selected patients.

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METFORMIN PROTECTS GLYCOGEN STORES IN LIVER AND SKELETAL MUSCLES AGAINST EXERCISE-INDUCED DEPLETION IN RATS

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Considering that hypoglycemic drugs may interact with exercise, we studied the effects of metformin (M) on glycogen storage (GLY) and glycemia in exercised rats. **Material and methods:** Three month old male Wistar rats were grouped (N= 4) as sedentary (S, SM), exercise-stressed (ES, ESM) and trained (T, TM). Training consisted of 12 swimming sessions of 50 min, with 5% of their body weight attached to the body. ES rats swam only one. MET (2.2 mg/kg/d) was administered in the drinking water. After the last session, samples were taken under pentobarbital anesthesia to measure glycemia (mg/dL) and GLY (mg/100 mg) in liver, soleus, gastrocnemius and left ventricle. Data (mean ± SE) were analyzed by two-way ANOVA ($\alpha = 0.05$). **Results:** Exercise increased glycemia and GLY breakdown in liver and skeletal muscles ($P<0.001$). MET increased GLY in liver and soleus in S group and. partially, protected these tissues and gastrocnemius in ESM group from high hyperglycemia. and related GLY depletion. Training increased GLY but, interaction with MET was only seen in soleus muscle ($P<0.05$). **Conclusion:** During exercise MET protected the reservoir of GLY in liver, soleus and gastrocnemius and lowered hyperglycemia. Interaction between training and metformin was demonstrated for glycemia and liver and soleus glycogen. ($P<0.05$, *ES vs S, # M vs Control, ♦ T,TM vs S, ES, ESM).

	LIVER	SOLEUS	GASTROC.	VENTRICLE	GLYCEMIA
S	3.85 ± 0.30	0.25 ± 0.01	0.30 ± 0.05	0.15 ± 0.01	118.8 ± 2.8
SM	5.17 ± 0.03#	0.34 ± 0.01#	0.38 ± 0.02	0.15 ± 0.01	123.2 ± 3.2
ES	1.00 ± 0.04*	0.14 ± 0.02	0.18 ± 0.03	0.12 ± 0.01	253.3 ± 4.6*
ESM	2.67 ± 0.10#	0.22 ± 0.02#	0.24 ± 0.02#	0.06 ± 0.01	183.6 ± 3.4#
T	5.99 ± 0.70♦	0.24 ± 0.04	0.53 ± 0.09♦	0.31 ± 0.03+	279.6 ± 8.3*
TM	5.56 ± 0.90♦	0.35 ± 0.03♦	0.45 ± 0.11♦	0.28 ± 0.02+	204.4 ± 40.8*

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EFFECT OF METFORMIN ON SERUM LEPTIN IN OBESE NIDDM PATIENTS

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Background: Leptin is a polypeptide hormone secreted by the adipocyte, and is one of the central regulators of body weight homeostasis. Human obesity and type-2 diabetes are associated with insulin resistance. Metformin in an antihyperglycemic agent which acts by improving insulin sensitivity and/or promoting modest weight loss. **Objective:** To explore insulin-leptin interrelation in the context of diabetic control under short-term use of metformin. **Study Design:** Prospective case-control study conducted on 20 obese women with NIDDM, treated for 1 week with metformin 1500 mg qd, without diet modification. Fasting and post-prandial blood glucose, and fasting plasma insulin and leptin were measured before and after treatment. Baseline parameters were measured in 2 control groups; 10 lean non-diabetic women and 10 obese non-diabetic women. **Results:** Fasting leptin was significantly higher in obese diabetics (24.87 ± 20.87 ng/ml) and obese non-diabetics (13.74 ± 10.65 ng/ml) compared to lean non-diabetics (2.74 ± 1.54 ng/ml)($p < 0.001$). Fasting insulin was significantly higher in obese diabetics (14.68 ± 7.14 mU/ml) and obese non-diabetics (7.00 ± 2.94 mU/ml) when compared with lean non-diabetics (4.75 ± 1.55 mU/ml)($p < 0.05$). There was a significant positive correlation between basal serum leptin and insulin ($r = 0.6166$, $p < 0.001$). Plasma leptin correlated positively with BMI ($r = 0.5954$, $p < 0.001$)). After one week of metformin treatment, obese diabetic women had significant improvement in glycemia and significant reduction of fasting serum insulin and leptin levels. Fasting plasma glucose dropped from 205.55 ± 89.77 mg/dl to 174.10 ± 85.69 mg/dl ($p < 0.001$). Postprandial plasma glucose dropped from 257.75 ± 98.44 mg/dl to 213.15 ± 99.72 mg/dl ($p < 0.001$). This was accompanied by lowering of basal plasma insulin from 14.68 ± 7.14 mU/ml to 10.53 ± 6.16 mU/ml ($p < 0.01$) and leptin from 24.87 ± 20.87 ng/ml to 15.09 ± 9.94 ng/ml ($p < 0.01$). **Conclusion:** 1) Human obesity in Egyptian individuals is a hyperleptinemic, leptin-resistant state. 2) Serum leptin positively correlates with BMI and serum insulin levels. 3) Metformin therapy in type-2 diabetes improves insulin sensitivity independent of weight reduction. 4) Metformin therapy is associated with reduction in serum leptin level independent of weight reduction. 5) To address leptin as a link between obesity and insulin resistance needs further exploration.

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PREVALENCE OF, AND RISK FACTORS FOR, HYPERLACTAEMIA IN METFORMIN-TREATED PATIENTS: THE FREMANTLE DIABETES STUDY.

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Aims: To assess the prevalence and effects of, and risk factors for, hyperlactaemia complicating metformin therapy in type 2 diabetes.

Materials and Methods: The fasting plasma lactate was measured in 184 metformin-treated patients with type 2 diabetes aged 37.5 to 83.8 years. All were participants in the Fremantle Diabetes Study, a prospective community-based study of diabetes care, control and complications, and represented approximately 10% of all patients with type 2 diabetes identified in a postcode-defined multi-ethnic urban population of 120,097.

Results: All patients were taking metformin in doses ranging from 250 mg to 5,100 mg daily. 31.5% were on metformin alone, 61.5% were also taking a sulphonylurea drug and 7.0% were on metformin and adjunctive insulin therapy with or without a sulphonylurea. The fasting plasma lactate (mean ± SD; 1.97 ± 0.70 mmol/L) was normal (<2.0 mmol/L) in 59.2% of the sample, between 2.0 and 3.0mmol/L in 34.2%, from 3.0 to 4.0 mmol/L in 5.4% and >4.0 mmol/L in 2 patients (1.1%). In a linear regression model with plasma lactate as the dependent variable, each of body mass index, plasma glucose, HbA_{1c} (inversely), serum creatinine >125 μmol/L and a metformin dose >1,500 mg/day were significantly and independently associated with the fasting plasma lactate ($P<0.032$). Age, duration of diabetes and use of diabetes treatment other than metformin were variables entered into, but excluded from, the model. There was a significant positive association between plasma lactate and pulse rate ($P=0.008$) and a weak inverse correlation with the serum bicarbonate ($P=0.063$).

Conculsions: 1. Hyperlactaemia is common in metformin-treated type 2 diabetic patients in the community and may have measureable effects. 2. Overweight, renal impairment and high doses of metformin are associated with hyperlactaemia. 3. Glycaemic control may have a complex relationship with plasma lactate. 4. Regular monitoring of plasma lactate may be advisable in patients receiving metformin regardless of age and concomitant antidiabetic therapy.

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EVALUATION OF LACTIC ACIDOSIS RISK IN PATIENTS TREATED WITH MAXIMUM DOSAGE OF METFORMIN

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Metformin is not only able to control glycaemic levels in type 2 diabetics, but also improves other factors of the insulin resistance syndrome. The risk of lactic acidosis has attracted new attention since its reintroduction in the American market. **Aims :** Evaluate changes in the acid-basis equilibrium and in the levels of lactacidaemia in obese patients treated with 2550 mg/day of metformin. **Materials and methods :** We compared two groups of obese patients, treated with maximum dosage of metformin, with a control group of 25 untreated obese non-diabetic patients : Group 1 - 22 obese patients (20 females, age 38±12 years, BMI=35.0±4.5 Kg/m^2, waist/hip ratio=0.85±0.08) ; Group 2 - 25 obese type 2 diabetics (20 females, age 55±9 years, BMI=34.1±5.2 Kg/m^2, waist/hip ratio=0.96±0.08) ; Group 3 - 25 untreated obese non-diabetic patients (21 females, age 38±9 years, BMI=36.5±7.1 Kg/m^2, waist/hip ratio=0.88±0.1). Patients with renal or hepatic pathology or with any condition which may cause hypoxia were excluded as well as women in their fertile age not using anticonceptional methods. Patients were treated for at least 3 (3 to 36) months and after 10 hours fasting, blood was collected in the radial artery and pH, lactate, anionic gap, bicarbonate, arterial blood pressures of carbon dioxide (pCO_2) and oxigen (pO_2) were determined. **Results :** We found no abnormal pH or lactate values in any patient. There were no significant differences between the 3 groups in pH or lactate but we found significant differences in anionic gap, bicarbonate, pO_2 and pCO_2. Group 2 had higher levels of bicarbonate ($p=0.01$), anionic gap ($p<0.005$) and pCO_2 ($p=0.05$) and lower levels of pO_2 ($p<0.0001$) than the other two groups; these parameters were correlated with age. No relationship was found between age or treatment duration and pH or lactate levels. **Conclusions:** We conclude that there is no significant risk of lactic acidosis during treatment with maximum dosage of metformin, provided that contraindications are duly observed.

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COMBINED METFORMIN AND INTENSIVE INSULIN THERAPY IN SEVERELY OBESE TYPE 2 DIABETIC PATIENTS

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Aims: We examined the effect of adjunct metformin on metabolic control, body weight and insulin secretion in severely obese type 2 diabetic patients pretreated with intensive insulin therapy. **Materials and Methods:** The study had a randomized, doubleblind crossover design. 13 obese type 2 diabetic patients (51 ± 9 years old, BMI 39 ± 3.9 kg/m²) in suboptimal glycaemic control pretreated with intensive insulin therapy were assigned to either metformin or placebo for 10 weeks after a two week run-in period. After a two week wash-out period they received the opposite treatment for 10 additional weeks. An oral glucose tolerance test with insulin and C-peptide determination was performed before and after each treatment period. **Results:** During metformin treatment HbA1c decreased from 8.5 ± 0.4 to 7.5 ± 0.3% (p=0.009) while during placebo HbA1c improved only marginally from 8.1 ± 0.4 to 7.6 ± 0.3% (p=0.18). Total exogenous insulin decreased from 53 ± 10 to 35 ± 7 U (p=0.006) during metformin treatment. Changes in fasting insulin levels during placebo and metformin were not different (p=0.11). Metformin had no effect on body weight and on serum triglycerides but decreased serum cholesterol levels from 239 ± 18 to 211 ± 14 mg/dl (p=0.005). During the oral glucose tolerance test the area under the curve for C-peptide increased during metformin (p=0.04) while no differences were observed for glucose and insulin. **Conclusions:** Addition of metformin to intensive insulin therapy in severely obese type 2 diabetic patients improves glycaemia but not hyperinsulinaemia in comparison to intensive insulin therapy alone. With adjunct metformin ~30% less exogenous insulin is required. With respect to glycaemia and lipids, adjunct metformin can be a reasonable treatment alternative in patients with type 2 diabetes already on intensive insulin therapy.

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METFORMIN'S BENEFICIAL EFFECT ON IMPAIRED GLYCOREGULATION IN CHRONIC GLUCOCORTICOID THERAPY

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Aim:To test the possible effect of metformin (M) on deviations in glycoregulation induced by glucocorticoid (Gc) administration. Patients, methods : 11 girls (16 –19 yrs old) on long-term Gc therapy for chronic connective tissue inflammation were examined, in addition to glucose tolerance, for hormones involved in glycoregulation before and 6 months after M administration (1000 mg/day). The daily dose of Gc, prior to the study, was 6.7 mg prednisone/day in average and 7.1 mg during the study. Results :

	Baseline	after M therapy	p
BMI,kg/m2	20.6+3.4	20.7+3.7	ns
HbAlc , % , Abbott IMx	6.9+1.2	6.1+0.6	ns
fasting glucose, mmol/l	4.3+ O.52	4.4+0.43	ns
fasting insulin, mIU/l, IRMA	12.8+14.5	8.5+5.3	< 0.05
fasting proinsulin, pmol/l, ELISA	3.37+2.46	1.25+0.94	< 0.001
fasting C-peptide, nmol/l, IRMA	0.82+0.41	0.85+0.47	ns
fasting glucagon, pmol/l, RIA	41.1+17.1	26.6+11.7	< 0.01
fasting insulin/fasting glucose (f i/f g)	2.98+1.32	1.93 +1.81	< 0.05
fasting insulin/fasting proinsulin (f i/f pi)	5.3+5.6	12.8+13.9	< 0.05
HOMA – resistence	2.40+2.54	1.68+1.12	< 0.05
HOMA –secretion	332+256	206+107	< 0.05

x + SD, Wilcoxon paired tests. Conclusions: Six-month administration of 1000 mg of M was associated with : a) a decrease in insulin resistance (IR) as documented by a significant fall in the fasting levels of insulin , proinsulin and also in the HOMA resistance and f i/f g indexes. The decrease in HOMA secretion and the significant rise in the calculated index f i/f pi during M therapy suggests a beneficial modulation of compensatory insulin hypersecretion while IR declined b) a marked decrease in fasting glucagon (which typically rises during Gc therapy). c) M was well tolerated and led to HbAlc normalization in all patients without a diet modifications.Supported by IGA grant 4849 - 3

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CONTINUOUS SUBCUTANEOUS INSULIN INFUSION IN TYPE 1 DIABETES : INSULIN-SPARING EFFECT OF METFORMIN.

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Aims : The effect of metformin (850 mg twice a day) was studied when added to insulin therapy in 62 patients previously treated for at least one year with continuous, subcutaneous insulin infusion (CSII). Methods : This study was a monocenter double blind placebo controlled parallel groups randomized trial of 6 months duration. Examinations took place every 2 months. Statistical analysis was performed using AnCova under the intention to treat principle. Results : Patients in the metformin group (M, n=31) were not significantly different from those in the placebo group (P, n=31) with regard to sex ratio: 14F/17M vs 11F/20M, age: 39.9±12 vs 41.1±10 years, BMI: 26.4±4.6 vs 25.8±3.6 kg/m^2, HbA1c: 7.5±0.8 vs 7.5±0.7% (mean±sd). At baseline, there was no difference in total insulin mean dose (M vs P): 0.73±0.20 vs 0.72±0.20 U/kg/day, basal insulin dose: 0.30±0.14 vs 0.27±0.08 U/kg/day, bolus dose: 0.41±0.13 vs 0.44±0.15 U/kg/day. Throughout the study period, BMI and HbA1c remained unchanged in both groups. Compared to baseline, total insulin dose at 6 months was reduced in M (mean-CI): -8.0% (-14 ; -2) vs +2.0% (0 ; +4.5) (p=0.005). Basal insulin dose was also reduced in M: -8.0% (-16 ; 0) vs +9.0% (0 ; +18) (p=0.015) whereas bolus dose was not significantly modified: -5.0% (-10 ; 0) vs + 0.0% (-7 ; +7) (p=0.08). Hypoglycemias (< 3.3 mM/L) were also reduced in M: 2.36±2.51 vs 3.26±2.75 events/patient/month (p=0.04). 3 patients in the metformin group presented minor digestive symptoms leading to interruption of the trial. Conclusion : These results suggest an insulin-sparing effect of metformin in type 1 diabetes mellitus treated with CSII.

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EFFECTS OF TROGLITAZONE AND METFORMIN ON GLUCOSE METABOLISM, PLASMA LEPTIN AND E-SELECTIN IN TYPE-2 DIABETES.

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Aims: Troglitazone (T) and metformin (M) have been shown to improve glucose control by increasing insulin sensitivity. We intended to compare the effects of these two drugs on insulin secretion and sensitivity, on body weight and plasma leptin, and on the endothelium specific soluble adhesion molecule E-Selectin in early Type-2 diabetes. **Methods:** Insulin secretion was evaluated by an oral glucose tolerance test (OGTT) combined with a mathematical model. 16 patients on diet therapy (age: 55.3± 2.0 yr; BMI: 27.5±1.9 kg/m^2; HbA1c: 8.5±0.3 %; WHR: 0.97±0.02; blood pressure: 122.3±5.1/79.5±4.1 mmHg) underwent a 75g 3hr OGTT for measurement of plasma glucose, insulin, proinsulin, C-peptide, leptin and plasma E-Selectin before and after administration of randomly given M (n=6; 1700 mg per day) or T (n=10; 600 mg per day). After 16 weeks of oral treatment, a second OGTT was performed to reevaluate glucose tolerance. Insulin sensitivity was determined by evaluating the dynamic area under the curve (AUC) of glucose and insulin, while B-cell secretion parameters were determined by analyzing data with a mathematical model (AJP 270: E522, 1996). **Results:** After treatment, insulin sensitivity improved as indicated by a marked reduction in glucose AUC (M: 45.0±6.4 vs. 53.4±5.9 M.3h, p=0.01; T: 41.9±3.4 vs. 51.2+4.0 M.3h, p=0.008) in spite of a similar insulin AUC (M: 36.6± 8.4 vs. 49.3± 14.1 nM.3h and T: 45.5±7.3 vs. 48.5±6.5 nM.3h, NS). Total insulin secretion and plasma proinsulin tended to decrease in all patients, while basal insulin secretion only decreased in patients on T (55.3±7.0 vs. 67.1±8.3 pM, p<0.03). Although no change in BMI and only a marginal decrease in plasma leptin (6.9±1.0 vs. 8.1±0.8 ng/ml, NS) was observed, WHR was slightly lower in both groups after therapy (0.93±0.002, p<0.05). E-Selectin decreased significantly (p<0.05) only in patients on T therapy (41.2±2.3 vs. 49.9±6.1 ng/ml). **Conclusions:** Both drugs improved glucose control to a similar extent without affecting body weight or plasma leptin. E-selectin was related to glucose metabolism and decreased in T after therapy potentially indicating improved endothelial function. As basal insulin secretion was only lowered in patients on T treatment, this drug seems to have a more pronounced effect on insulin sensitivity, while M might also act by other mechanisms.

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Oral Drugs: Thiazolidinedione Derivatives I

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LOW-DOSE ROSIGLITAZONE ENHANCES GLYCEMIC CONTROL WHEN COMBINED WITH SULFONYLUREAS IN TYPE 2 DIABETES

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Aims: To investigate the efficacy of low doses of rosiglitazone (RSG) when added to sulfonylurea (SU) therapy in type 2 diabetes (T2D). **Materials and Methods:** Six-month, randomized, double-blind study of 574 T2D patients receiving RSG (2 or 4mg as 2 divided doses) or placebo (PBO), in addition to SU. All patients had been taking gliclazide, glibenclamide, or glipizide for ≥6 mos. **Results:** RSG produced clinically and statistically significant reductions in HbA1c and FPG, with greatest effects at 4mg/d. Improvements in HbA1c were the same, regardless of the SU used. Decreases in FFA (c.15%) and increases in HDL (c.10%) and LDL (c.5%) were seen with RSG 4mg/d. The overall incidence of adverse events was similar in all 3 groups, with no significant hypoglycemia or hepatotoxicity. **Conclusions:** RSG 4mg/d is safe, well tolerated, and effective when added to SU therapy. Its benign safety profile at 4mg/d supports the investigation of higher doses of RSG in combination with SUs.

	SU Alone (n=192)	SU + RSG 2mg/d (n=199)	SU + RSG 4mg/d (n=183)
HbA1c (%)			
Mean baseline	9.2	9.2	9.2
Mean Δ from baseline±SD	+0.2±1.11	-0.5*±1.05	-0.9*±1.10
Difference from SU alone	NA	-0.6*	-1.0*
% with reduction ≥0.7%	19%	39%†	60%†
FPG (mmol/L)			
Mean baseline	11.5	11.3	11.4
Mean Δ from baseline±SD	0.3±2.7	-0.9*±2.7	-2.1*±2.6
Difference from SU alone	NA	-1.3*	-2.4*
% with reduction ≥1.7mmol/L	21%	38%†	56%†

*P<0.0001; †P<0.0001 for comparison with SU alone

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ROSIGLITAZONE DOES NOT MARKEDLY ALTER CYP3A4-MEDIATED DRUG METABOLISM

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Aims: Over 150 drugs are known to be metabolized by CYP3A4. The effect of therapeutic doses of RSG, a potent thiazolidinedione, on the pharmacokinetics (PK) of three CYP3A4-metabolized drugs—nifedipine (N) and the oral contraceptive (OC) constituents ethinylestradiol (EE) and norethindrone (NE)—was studied in 2 placebo (PBO)-controlled studies. **Materials and Methods:** Studies followed a randomized, cross-over design. In the OC study, 34 women received 2 consecutive cycles of OC (EE 0.035mg, NE 1mg) with concomitant RSG 8mg once daily (od) or PBO for the first 14 days of each cycle. PK sampling was performed on day 14. In the N study, 28 men received a single oral dose of N (20mg) or RSG 8mg od x 14d + a single dose of N on day 14. PK sampling was performed over the 24 hours following the N dose. Dosing cycles were separated by a 14-day washout period. Lack of PK effect was prospectively defined if the 90% confidence interval (CI) was contained within a predefined equivalence range. **Results:** Coadministration of RSG resulted in no clinically significant changes in the PK of EE, NE, or N. **Conclusions:** RSG does not significantly alter the metabolism of N or the OC constituents EE and NE. Thus, RSG is not expected to interact significantly with drugs metabolized by the common CYP3A4 pathway. This further supports the results found in rosiglitazone *in vitro* and animal studies.

Mean (SD) Pharmacokinetic Parameters

Ethinylestradiol	**RSG + OC**	**PBO + OC**	**PE (90% CI)**
AUC(0-24) (pg·h/ml)	1126 (386)	1208 (404)	0.92 (0.88, 0.97)
Cmax (pg/ml)	123 (42)	130 (47)	0.95 (0.88, 1.02)
Norethindrone	**RSG + OC**	**PBO + OC**	**PE (90% CI)**
AUC(0-24) (pg·h/ml)	178 (67)	171 (62)	1.04 (1.00, 1.07)
Cmax (pg/ml)	21.5 (6.7)	22.1 (6.8)	0.97 (0.91, 1.03)
Nifedipine	**N**	**RSG +N**	**PE (CI)**
AUC(0-∞) (ng·h/ml)	338(135)	298(110)	0.87 (0.79, 0.96)†
Cmax (ng/ml)	137±86	138±88	0.99 (0.68, 1.43)‡

†90% CI; ‡95% CI

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THE EFFECT OF PIOGLITAZONE ON GLUCOSE CONTROL AND LIPID PROFILE IN PATIENTS WITH TYPE 2 DIABETES

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Aims: To study the effect of pioglitazone (PIO), a new thiazolidinedione, on glucose control and lipid profile in patients with type 2 diabetes. **Materials and Methods:** This was a multicentre, double-blind, placebo-controlled study. Patients entered a 1-week screening period followed by a 5-week single –blind placebo lead in. Patients were then randomised to PIO 30 mg (n=101) or placebo (n=96) for 16 weeks. Baseline values, least square mean change from baseline (Δ baseline) and least square mean change from placebo (Δ placebo) are reported. Responders were defined as ≥0.6% decrease in HbA1c or decrease in fasting blood glucose (FBG) of ≥30 mg/dl. **Results:**

	Treatment	Baseline	Δ baseline	Δ placebo
HbA1c (%)	P	10.28	0.76*	-
	PIO	10.54	- 0.60*	1.37**
FBG (mg/dl)	P	270.1	7.7	-
	PIO	272.6	-49.8*	-57.5**
Triglyceride (TG) (mg/dl)	P	335.1	-18.5	-
	PIO	400.4	-103.8*	-85.2**
HDL-cholesterol (mg/dl)	P	39.3	0.3	-
	PIO	37.7	5.3*	5.0**

*p≤0.05 vs baseline (paired t-test); **p≤0.05 (Dunnett's test)

PIO significantly reduces HbA1c and FBG. Furthermore a greater number of PIO patients were classified as responders in terms of HbA1c (48% vs 11%) and FBG (61% vs 23%). In addition fasting TG levels were significantly decreased while HDL-cholesterol levels were significantly increased in response to PIO. Total and LDL-cholesterol levels were not significantly different from placebo. **Conclusions:** PIO monotherapy improves glycaemic control and lipid profile in patients with type 2 diabetes.

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ROSIGLITAZONE IS EFFECTIVE AND WELL TOLERATED IN PATIENTS ≥65 YEARS WITH TYPE 2 DIABETES

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Aims: To review evidence of the efficacy and safety of rosiglitazone (RSG) in type 2 diabetes mellitus (T2DM) patients ≥65 years of age. **Materials and Methods:** Pooled data from two randomized, double-blind, placebo (PBO)-controlled studies of RSG as monotherapy for T2DM. **Results:** In two 26-week trials, RSG 4mg/d and 8mg/d produced similar treatment effects on fasting plasma glucose (FPG) and HbA1c in patients <65 years and in those ≥65 years, with decreases up to 4.4 mmol/L FPG and up to 1.6 percentage points HbA1c in patients receiving 8mg/d. Of 2,526 patients who have received RSG monotherapy in double-blind studies, 33% were ≥65 years. Among these patients, the incidence of adverse events (AE) was similar to that reported in patients <65 years. Edema occurred in 3.5% of patients <65 years and 7.5% of patients ≥65 years on RSG alone and in up to 1.7% of patients on PBO. Anemia occurred in 1.7% and 2.5% of patients <65 and ≥65 years, respectively, on RSG alone and in up to 1% of patients on PBO. Hypoglycemia occurred in <1% of patients on RSG monotherapy, regardless of age. **Conclusions:** RSG monotherapy is effective, safe, and well tolerated in older patients with T2DM.

	Incidence of Adverse Events			
	RSG Monotherapy		Placebo	
Adverse Event	<65 (n=1694)	≥65 (n=832)	<65 (n=404)	≥65 (n=197)
Upper respiratory infection	11.1%	7.6%	9.4%	7.1%
Injury	7.7%	7.5%	4.7%	3.6%
Urinary tract infection	3.2%	4.9%	2.7%	3.6%
Back pain	3.7%	4.7%	3.2%	5.1%
Hyperglycemia	3.7%	4.4%	5.4%	6.1%
Headache	6.7%	4.2%	5.0%	5.1%

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PIOGLITAZONE HAS LOW POTENTIAL FOR DRUG INTERACTIONS

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Aim: Pioglitazone (PIO) is an effective antihyperglycaemic agent for the treatment of type 2 diabetes. It is highly protein bound to albumin. In vitro studies have shown no inhibition of cytochrome P450 isoenzyme by PIO. The aim of this study was to investigate the influence of PIO on the pharmacodynamics and pharmacokinetics of drugs commonly prescribed for the treatment of type 2 diabetes, and associated complications. **Materials and Methods:** PIO (single and repeat doses of 45 mg/day) was co-administered with warfarin, phenprocoumon, glipizide, metformin or digoxin in healthy volunteers. **Results:** PIO caused no significant pharmacokinetic or pharmacodynamic changes in the co-administered drug. Furthermore, co-administration of PIO with any of these drugs was safe and well tolerated. PIO undergoes extensive hepatic metabolism by hydroxylation of aliphatic methylene groups, predominantly via the cytochrome P450 isoenzymes CYP3A4, CYP2C8/9 and CYP1A1/2. R-warfarin and its active enantiomer S-warfarin are metabolised by CYP1A1/2 and CYP2C9, respectively. The pharmacokinetic profiles of R-warfarin and S-warfarin were unaffected by repeat doses of PIO. This suggests that the drug neither inhibits nor induces these P450 enzymes. Exposure of healthy volunteers to PIO had no effect on urinary 6-beta-hydroxycortisol/cortisol ratio (a generally accepted marker of CYP3A4), suggesting that CYP3A4 is not induced. **Conclusion:** These data suggest PIO does not effect the pharmacokinetics and pharmacodynamics of warfarin, phenprocoumon, glipizide, metformin or digoxin, and does not appear to induce or inhibit the P450 isoenzyme system. PIO has only a very small potential for drug interactions in patients with type 2 diabetes.

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ROSIGLITAZONE IS EFFECTIVE IN BOTH OBESE AND NON-OBESE PATIENTS WITH TYPE 2 DIABETES

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Aims: To evaluate the efficacy of rosiglitazone (RSG) in obese and non-obese patients with type 2 diabetes (T2D). **Materials and Methods:** Data was pooled from 3 multicentre, double-blind studies of RSG used as monotherapy for T2D, in which a total of 1985 patients previously treated by diet and exercise, or oral hypoglycaemic therapy, had evaluable fasting plasma glucose (FPG) & Body Mass Index (BMI). In these studies, patients had been randomised to receive: RSG 4mg, 8mg or glibenclamide (glyburide) (1 study); RSG 4mg, 8mg or placebo (2 studies). The change from baseline in FPG after 6 months' treatment was analysed according to BMI category (obese: BMI $\geq 27 kg \cdot m^{-2}$; non-obese <27). **Results:** Are tabulated below:

Change in FPG (mM) after 6 months' treatment (last observation carried forward)				
	Obese (BMI ≥ 27 kg·m^{-2})		Non-obese (BMI <27 kg·m^{-2})	
	N	Mean ± SE	N	Mean ± SE
Placebo	227	+0.76 ± 0.20	103	+0.67 ± 0.33
RSG 4mg/day	532	-2.04 ± 0.12	195	-1.34 ± 0.19
RSG 8mg/day	515	-2.86 ± 0.13	210	-2.46 ± 0.18
Glibenclamide	124	-2.51 ± 0.22	79	-2.52 ± 0.26

Conclusions: Although insulin resistance tends to be greater in obese patients, these results demonstrate that the insulin sensitiser RSG is effective in improving glycaemic control both in obese and non-obese patients with type 2 diabetes.

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GENDER DIFFERENCE IN PROMOTION OF SUBCUTANEOUS FAT ACCUMULATION WITH LONG-TERM TROGLITAZONE TREATMENT

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Aims: We recently reported that troglitazone promoted fat accumulation in subcutaneous rather than visceral adipose tissue. On the other hand, it is well known that there is gender difference in body fat distribution. In the present study, we examined gender difference in the effect of troglitazone on body fat distribution. Materials and Methods: Forty-one type 2 diabetes patients with poor glycemic control , were administered troglitazone at a dose of 400 mg/day for 12.5 ± 5.5 months. At the start of troglitazone treatment, 22 (eight male and 13 female) patients were treated by diet alone (T group) , and the others (10 males and nine females) with glibenclamide at a dose of 2.5 to 7.5 mg/day (SU+T group). BMI, HbA1c and body fat distribution, determined by abdominal CT at the umbilical level, were compared before and after troglitazone treatment. Results: With troglitazone treatment, HbA1c levels decreased and BMI increased in both genders of the T group and in females of the SU+T group. Concerning body fat distribution in the T group, visceral fat area (VFA) was unchanged, subcutaneous fat area (SFA) increased siginificantly in males (138.5 ± 68.0➡163.6 ±78.6, $p<0.05$) and in females (235.7±83.6➡284.2 ± 100.2, $p<0.0001$). The increase in SFA was more marked in females than in males. In the SU+T group, VFA was unchanged, SFA increased siginificantly only in females (224.1 ± 116.8➡270.8 ± 145.4, $p<0.05$). Significant correlations between the decrease in HbA1c and the increase in BMI and SFA were observed in this group. Conclusions: Sex hormone might contribute to promotion of subcutaneous fat accumulation with troglitazone and to troglitazone-mediated amelioration of insulin resistance.

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ROSIGLITAZONE DECREASES INSULIN RESISTANCE AND IMPROVES BETA-CELL FUNCTION IN PATIENTS WITH TYPE 2 DIABETES

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Aims: To assess the effect of rosiglitazone, a potent member of the thiazolidinedione class of drugs, on insulin resistance and beta-cell function in patients with type 2 diabetes mellitus. **Materials and Methods:** Using data from three trials of rosiglitazone as mono- or combination therapy, a homeostasis model assessment (HOMA) was performed using fasting plasma glucose and insulin following 26 weeks' treatment. The effect of rosiglitazone administered alone or in combination with sulfonylureas (SU) or metformin (MET) was determined. **Results:** Rosiglitazone alone or in combination with SU or MET significantly reduced insulin resistance relative to baseline whilst improving beta-cell function. In contrast, there was a worsening of both indices in patients treated with placebo, and insulin resistance increased in patients treated with SU. In addition, MET alone did not improve insulin resistance or beta-cell function. **Conclusions:** Rosiglitazone monotherapy or in combination with SUs or MET decreased insulin resistance and improved beta-cell function in patients with type 2 diabetes mellitus.

Regimen, *n*	**Mean Change in Insulin Resistance*(%)**	**Mean Change in Beta-Cell Function*(%)**
Placebo, *158*	+7.9 (*P*=.078)	-4.5 (*P*=.32)
RSG 2mg/bd, *166*	-16.0 (*P*<.00001)	+49.5 (*P*<.0001)
RSG 4mg/bd, *169*	-24.6 (*P*<.00001)	+60.0 (*P*<.0001)
SU, *192*	+15.0 (*P*<.04)	+8.6 (*P*<.04)
RSG 1mg/bd+SU, *198*	-2.6 (*P*<.50)	+22.8 (*P*<.0001)
RSG 2mg/bd+SU, *183*	-17.4 (*P*<.00001)	+72.0 (*P*<.0001)
MET, *113*	+0.46 (*P*=.89)	+0.75 (*P*=.85)
RSG 4mg/od+MET, *115*	-3.2 (*P*=.75)	+40.3 (*P*=.0018)
RSG 8mg/od+MET, *109*	-20.4 (*P*=.0017)	+94.2 (*P*=.04)
*at week 26; RSG: rosiglitazone		

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ROSIGLITAZONE REDUCES PLASMA INSULIN & ITS PRECURSORS WHILE DECREASING GLYCAEMIA IN TYPE 2 DIABETICS

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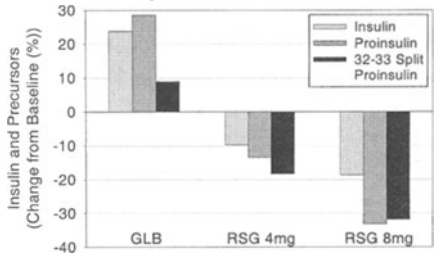
Aims: Examine the effects of rosiglitazone (RSG) on insulin, proinsulin and 32-33 split proinsulin, which are disproportionately elevated in type 2 diabetes and which are indicative of β-cell dysfunction. **Materials and Methods:** RSG (4mg and 8mg, given in 2 divided doses) was compared to optimally titrated glibenclamide (glyburide) (GLB) in 587 type 2 diabetics in this 12-month randomised, double-blind study. **Results:** RSG 4mg (-6.2pmol/L) and 8mg (-12.5pmol/L) reduced mean plasma insulin (both $p<0.0001$), whereas GLB (+14.6pmol/L) increased plasma insulin ($p<0.0001$) compared with baseline. All three treatments decreased mean fasting plasma glucose: -1.4, -2.3 and -1.7mmol/L for RSG 4mg, 8mg and GLB, respectively (each $p<0.0001$ compared with baseline). RSG 8mg was superior to GLB in lowering FPG ($p<0.05$) and equivalent to GLB in reducing HbA1c. RSG also reduced the insulin precursors proinsulin (-2.9 & -4.6pmol/L for RSG 4mg & 8mg, respectively; each $p<0.0001$) and 32-33 split proinsulin (-5.0 & -9.1pmol/L respectively; each $p<0.0001$), and these decreases were proportionally greater than the reductions in insulin. GLB increased proinsulin (+5.0pmol/L; $p<0.0001$) and 32-33 split proinsulin (+2.9pmol/L; $p<0.001$), and these were both significantly different from the two RSG groups (each $p<0.0001$). **Conclusions:** As well as improving glycaemic control, RSG causes a significant dose-related reduction in insulin and proportionally greater reductions in proinsulin and 32-33 split proinsulin, whereas GLB increases these parameters. These results indicate that the insulin sensitiser RSG may reduce β-cell stress while decreasing insulin resistance.



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A WEAK PPARγ ACTIVATING THIAZOLIDINEDIONE WITH POTENT ANTIDIABETIC AND HYPOLIPIDEMIC ACTIVITIES

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Aim: This study describes the insulin sensitizer and lipid lowering activities of a novel pyridine analogue of thiazolidinedione, PAT5A in different animal models. **Materials and Methods**: The insulin sensitizer potential of PAT5A was tested in genetic animal models namely C57BL/KsJ-db/db and C57BL/KsJ-ob/ob mice. High fat fed Sprague Dawley rats were used for assessing the lipid lowering activities. *In vitro* transactivation studies were performed using HEK293 cells. Twenty-eight days probe toxicity study was conducted in Wistar rats. **Results**: Administration of PAT5A in db/db mice for 15 days showed dose dependent decrease in plasma glucose, triglyceride, insulin and improvement in glucose tolerance. In this model PAT5A showed better activity than troglitazone and comparable glucose lowering activity to rosiglitazone. However, the lipid lowering activity of PAT5A was better than the standard compounds. In ob/ob mice model, administration of PAT5A for 14 days showed dose dependent reduction in plasma glucose, triglyceride and insulin levels. A significant improvement in glucose tolerance was also observed. In high fat fed rats PAT5A administered at 10 mg/kg for 3 days showed 38 and 30% reduction in plasma triglyceride and total cholesterol levels respectively. Both troglitazone and rosiglitazone failed to show any effect in this animal model. In *in vitro* PPARγ transactivation assay, PAT5A showed poor transactivation potential. It did not show any PPARα or δ activating property. db/db animals treated with PAT5A showed decreased liver glucose 6-phosphatase activity, a key enzyme in gluconeogenesis. In 28 days probe toxicity studies in rats at 50 mg/kg dose, no treatment related alterations in hematological parameters or any macroscopic and microscopic changes were observed in the vital organs whereas rosiglitazone treatment showed increase in liver and heart weight. **Conclusion**: Our results indicate that PAT5A is a potent insulin sensitizer and lipid lowering compound. The compound showed a poor PPAR transactivation potential. Therefore, the antidiabetic activity of PAT5A could be mechanistically distinct from other thiazolidinediones and will be devoid of PPAR related toxicity.

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Oral Drugs: Thiazolidinedione Derivates II

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ROSIGLITAZONE IS EFFECTIVE IN POORLY CONTROLLED TYPE 2 DIABETES PATIENTS

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Aims: To evaluate the efficacy of rosiglitazone (RSG) in patients with poorly controlled type 2 diabetes (DM) (HbA1c ≥9% at baseline), those who are at greatest risk for microvascular complications of DM. **Materials and Methods:** Pooled data from 3 multicentre, double-blind studies of RSG as monotherapy for DM. A total of 2090 patients were randomized to receive: RSG 2mg/bd, 4mg/bd or glibenclamide for 52 weeks (n=598); RSG 4mg/od, 8mg/od, 2mg/bd, 4mg/bd or placebo (PBO) for 26 weeks (n=959); or RSG 2mg/bd, 4mg/bd or PBO for 26 weeks (n=533). Twenty six-week and baseline data were available for 758 patients with baseline HbA1c ≥9%. **Results:** RSG significantly improved glycemic control in patients with high baseline HbA1c, as indicated by significant reductions in HbA1c and FPG. Across all patient groups, RSG significantly reduced free fatty acids (FFA), with the greatest reduction observed in patients receiving 4 mg/bd ($p<0.0001$). **Conclusions:** RSG significantly improves glycemic control in DM patients with elevated HbA1c, potentially reducing the risk of microvascular complications in these patients. The dose-related response demonstrated in these studies indicates that RSG can be titrated to achieve optimal glycemic control in patients with DM.

	Mean Change at Week 26			
Regimen (n)	HbA1c% vs. baseline	HbA1c% vs. PBO	FPG (mmol/L) vs. baseline	FPG (mmol/L) vs. PBO
PBO (154)	0.84	--	0.51	--
RSG 4mg/od (91)	-0.05	-1.07	-1.59	-2.21
RSG 8mg/od (81)	-0.53	-1.44	-3.06	-3.42
RSG 2mg/bd (215)	-0.39	-1.42	-2.68	-3.44
RSG 4mg/bd (217)	-0.83	-1.84	-3.66	-4.63

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EFFICACY OF TROGLITAZONE IN TYPE 2 DIABETIC PATIENTS ADEQUATELY CONTROLED WITH GLIBENCLAMIDE

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Aims: The high dose administration of glibenclamide (G) may result in the exhaustion of pancreatic beta cell function. We examined the possibility of dose reduction of G in the treatment of type 2 diabetes by taking G together with troglitazone, and investigated changes of insulin secretion, fat accumulation and distribution. **Methods:** Sixty one patients with type 2 diabetes adequately controled (HbA1c 6.8±0.4%) with glibenclamide were randomly divided into troglitazone (400mg/day)-added (T) group (n=30) or control (C) group (n=31) and followed for 24 weeks. G dose was adjusted to equally maintain HbA1c level. Fat accumulation to the liver was evaluated by mean hounsfield unit of liver (LmHU). Subcutaneous fat accumulation (S) and body fat composition (BF%) were measured by the abdominal CT scan and bio-electrical impedance analysis method respectively. **Results:** There were no significant differences in body mass index, BF%, fasting plasma glucose and HbA1c in both groups after 24 weeks. In T group, G dose (1.5±1.7mg/day), serum fasting insulin (FIRI) (6.1±2.6μU/ml), fasting CPR (FCPR) (1.9±0.8ng/ml) and ALT (21.2±13.2IU/l) significantly ($p<0.05$) decreased in comparison with G dose (3.7±2.3mg/day), FIRI (9.0±6.46μU/ml), FCPR (2.6±1.38ng/ml) and ALT (28.0±13.7IU/l) before the treatment. In C group, G dose, FIRI, FCPR and ALT did not significantly change. In T group, LmHU and S after the treatment increased in comparison with those before the treatment. **Conclusion:** The additional administration of troglitazone decreased daily doses of glibenclamide, preserved fasting insulin secretion and improved fatty liver. This regimen also evaded body weight gain.

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PHARMACOKINETICS OF PIOGLITAZONE IN PATIENTS WITH RENAL IMPAIRMENT

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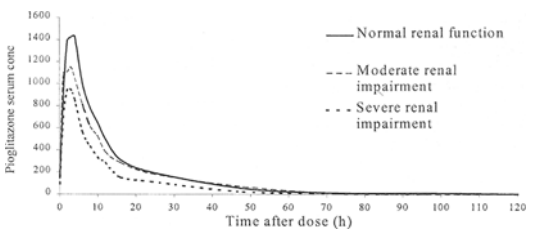
Aims: Renal impairment is a common complication of type 2 diabetes. The aim of this study was to investigate the pharmacokinetics of pioglitazone, an effective antihyperglycaemic agent for the treatment of type 2 diabetes, in subjects with impaired renal function. **Materials and Methods:** In this open label study, subjects with severe (creatinine clearance [CL_{CR}] <30 ml/min) (n=12) or moderate renal impairment (CL_{CR}= 30-60 ml/min) (n=9) and healthy controls (n=6) received pioglitazone 45 mg/day as single or repeat doses. **Results:** AUC of pioglitazone following single and repeat (see figure) dosages was reduced with increasing renal impairment. The same was true for the major metabolites of pioglitazone, MIII and MIV, suggesting an increased clearance of pioglitazone in patients with renal impairment. This may be explained by reduced protein binding resulting in an increase in the free fraction of pioglitazone. Since elimination of pioglitazone is via hepatic metabolism, any tendency to reduced protein binding will lead to increased hepatic clearance, with a resultant unchanged free drug concentration. **Conclusion:** Under these circumstances, dose adjustment of pioglitazone is not necessary for the treatment of diabetic patients with renal failure.



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ROSIGLITAZONE IN COMBINATION WITH METFORMIN EFFECTIVELY REDUCES HYPERGLYCEMIA IN PATIENTS WITH TYPE 2 DIABETES

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Aims: To investigate the efficacy of rosiglitazone (RSG) as combination therapy in type 2 diabetes (T2D) patients inadequately controlled with metformin (MET). **Materials and Methods:** Three hundred forty eight patients inadequately controlled on MET 2.5g/d (mean baseline FPG over 11.7mmol/L) were randomly assigned to receive MET 2.5g/d plus RSG 4mg/od, RSG 8mg/od, or placebo (PBO) for 26 weeks. **Results:** Fasting plasma glucose (FPG) and HbA1c decreased significantly in both RSG treatment groups, without increases in endogenous insulin levels. Thirty percent of patients receiving RSG 8mg/d and 22% of those receiving RSG 4mg/d achieved FPG of <7.8mmol/L, compared to 8% of PBO patients. LDL and HDL cholesterol increased in all treatment groups; however, total cholesterol/HDL ratio did not change significantly in any group. Free fatty acids decreased significantly in both RSG groups, but not in the PBO group. The incidence of adverse events was similar in all 3 groups, with no significant hepatotoxicity. **Conclusions:** RSG in combination with MET is significantly more effective at reducing hyperglycemia in T2D patients than MET alone.

	MET+PBO (n=113)	MET+RSG 4mg/od (n=116)	MET+RSG 8mg/od (n=110)
HbA1c (%)			
Baseline	8.6 ± 1.3	8.9 ± 1.3	8.9 ± 1.5
change from baseline	+0.45 ± 1.2	–0.56 ± 1.3*	-0.78 ± 1.2*
Comparison with MET	--	-0.97*	-1.18*
FPG (mmol/L)			
Baseline	11.9 ± 2.9	11.9 ± 3.2	12.2 ± 3.05
change from baseline	+0.3 ± 2.5	-1.8 ± 2.6*	-2.6 ± 2.9*
Comparison with MET	--	-2.2*	-2.9*

*P<0.0001; all values mean ± SD

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ROSIGLITAZONE PRODUCES LONG-TERM REDUCTIONS IN URINARY ALBUMIN EXCRETION IN TYPE 2 DIABETES

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Aims: Microalbuminuria (MA; [≥30, <300µg/mg]) portends adverse cardiovascular and renal risk for patients with type 2 diabetes mellitus (T2DM). The effect of rosiglitazone (RSG), a thiazolidinedione that improves insulin sensitivity and glycemic control in T2DM, on urinary albumin excretion (UAE) was examined as a secondary endpoint in a 52-week, open-label, glyburide (G)-controlled cardiac safety study. **Materials and Methods:** Patients (n=203) were randomly assigned to receive G or RSG 4mg twice daily (bd). G was titrated (mean dose 10.5 mg/day) to achieve optimal glucose control at the investigators' discretion for the first 8 weeks, after which the dose was held constant. The albumin/creatinine ratio (ACR), a measure of UAE, was determined from a random morning urine sample at baseline and following 52 weeks' treatment with RSG or G. From baseline to week 52, RSG-treated patients showed a statistically significant greater reduction in FPG compared to G (-3.61mmol/L vs –3.11mmol/L; p<0.006), although mean ΔHbA1c was similar (-0.9±1.4% for both groups). ΔACR was assessed using all randomized patients with baseline and week 52 ACR values (i.e. includes MA, macroalbuminuria [MCA] and normoalbuminuria [NA]). **Results:** Compared to baseline, RSG-treated patients with baseline MA (n=14) showed a Δgeometric mean ACR of -53.7% (-77.8%, -3.4%). By comparison, G treatment (n=16) resulted in a Δgeometric mean ACR of -22.6% (-43.1, 5.1). The proportion of patients with MA and MCA decreased from 24.6% to 19.3% during RSG treatment compared to an increase from 26.6% to 32.8% during G treatment. **Conclusion:** In addition to lowering glucose, RSG reduces urinary albumin excretion and could be of value in the treatment or prevention of microvascular and macrovascular complications of T2DM.

Treatment group (ACR = µg/mg)	Baseline ACR*	Comparison to Baseline (%Change)†	Comparison to G (%Difference)†
Glyburide (n=64)	16.1	-9.1 (-20.9, 4.6)	---
RSG 4mg bd (n=57)	16.1	-26.4 (-44.3, -2.7)	-19.0 (-38.9, 7.4)**

*geometric mean; †geometric mean of ratio (95% confidence intervals); **p=0.14

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ROSIGLITAZONE REDUCES URINARY ALBUMIN EXCRETION IN TYPE 2 DIABETES

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Aims: For patients with type 2 diabetes mellitus (DM), microalbuminuria (MA) is associated with a 4- to 6-fold risk of cardiovascular mortality and may herald progression to end-stage renal disease. Rosiglitazone (RSG), the most potent of the thiazolidinediones, improves insulin resistance and glycemic control when used alone or in combination with SUs, metformin, or insulin. The purpose of this study was to determine the effect of RSG on urinary albumin excretion (UAE). **Materials and Methods:** The effect of RSG on UAE (as measured by urinary albumin:creatinine ratio [ACR]) was examined as a secondary endpoint in 2 randomized, double-blind, placebo (PBO)-controlled monotherapy trials. Following a 4-week PBO run-in, RSG was administered for 26 weeks. ACR was measured from a random morning urine sample at baseline and following 26 weeks of treatment with RSG or PBO. ACR was assessed using analysis of covariance following log-transformation using the intent-to-treat population (i.e. patients with normoalbuminuria and MA) with last observation carried forward. **Results:** The subgroup of patients with baseline MA (ACR ≥30, <300 µg/mg; range of geometric means 68.7-84.8) were analyzed separately. Relative to PBO (n=57), RSG-treated patients (n=212) showed decreases in ACR ranging from –19% to –28%. **Conclusions:** Treatment with RSG reduces albumin excretion and could be of value in the treatment or prevention of secondary complications of DM.

Treatment group (ACR = µg/mg)	Baseline ACR*	Comparison to Baseline (%Change)$	Comparison to PBO (%Difference)$#
Study 011			
Pbo (n=132)	17.8	+3.6 (-9.1, 18.0)	---
RSG 2mg bd (n=142)	21.0	-14.0 (-25.3, -0.9)	-14.5 (-30.1, 4.6)
RSG 4mg bd (n=145)	16.0	-21.6 (-30.6, -11.3)†	-25.7 (-39.2, -9.2)††
Study 024			
Pbo (n=137)	14.6	-1.6 (-11.6, 9.6)	---
RSG 4mg od (n=152)	15.3	-4.0 (-14.3, 7.5)	-1.7 (-17.8, 17.5)
RSG 2mg bd (n=156)	15.9	-15.3 (-25.0, -4.3)	-12.7 (-26.9, 4.2)
RSG 8mg od (n=161)	16.9	-7.1 (-17.2, 4.3)	-3.3 (-18.9, 15.3)
RSG 4mg bd (n=155)	14.4	-12.1 (-21.2, -1.9)	-10.8 (-25.3, 6.5)

*geometric mean; $geometric mean of ratio; #Dunnett test; †p<0.001; ††p<0.005

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ROSIGLITAZONE GIVEN ONCE OR TWICE DAILY IS EFFECTIVE FIRST-LINE TREATMENT FOR TYPE 2 DIABETES MELLITUS

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Aims: To investigate the effect of rosiglitazone (RSG), a potent PPARγ agonist, on glycemic control in type 2 diabetes mellitus. **Materials and Methods:** Pooled data from 3 controlled trials of RSG as monotherapy for type 2 diabetes mellitus: 2 placebo (PBO)-controlled, 1 with an active comparator. In two 26-week, randomized, double-blind studies, patients received RSG at total daily doses of 4mg or 8mg (once daily [od] or divided [bd]), or PBO. Patients had been previously treated with diet alone or oral antidiabetic agents. At baseline (BL), mean fasting plasma glucose (FPG) and HbA1c for the aggregate study population were 12.5mmol/L and 8.9%, respectively. **Results:** FPG decreased significantly in all RSG treatment groups, without concomitant increases in serum insulin. RSG reduced HbA1c up to 1.5 percentage points relative to PBO; RSG 4mg/bd produced the greatest decreases. The incidence of adverse events was similar in all treatment groups, including placebo, and there was no evidence of hepatotoxicity. RSG produced clinically significant effects on FPG and HbA1c, in both prior diet and prior monotherapy subsets. **Conclusions:** RSG at total daily doses of 4mg and 8mg, administered once daily or as a divided dose, was well tolerated and significantly improved glycemic control in patients with type 2 diabetes mellitus.

Treatment Group (n)	BL FPG (mmol/L)	Δ BL	Difference from PBO*	% w/FPG <7.8mmol/L†
PBO (158)	12.7	1.0	–	3
RSG 2mg/bd (166)	12.6	2.1‡	3.2‡	25
RSG 4mg/bd (169)	12.2	3.0‡	4.2‡	39
PBO (173)	12.4	0.4	–	5
RSG 4mg/od (180)	12.7	1.4§	1.7§	17
RSG 2mg/bd (186)	12.5	1.9§	2.4§	25
RSG 8mg/od (181)	12.7	2.3§	2.7§	29
RSG 4mg/bd (187)	12.7	3.0§	3.4§	35

*Adjusted mean difference; †At week 26; ‡P=0.0001; §P<0.0001

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ROSIGLITAZONE IS AN EFFECTIVE ALTERNATIVE TO GLIBENCLAMIDE AS FIRST-LINE THERAPY IN TYPE 2 DIABETIC PATIENTS

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Aims: Rosiglitazone (RSG) is an effective treatment of type 2 diabetic patients when given once or twice daily. The efficacy of RSG in a sub-group of patients treated by diet and exercise alone prior to entry into this comparator study with glibenclamide (glyburide) (GLB) was examined. **Materials and Methods:** Efficacy of RSG (4mg and 8mg, given in 2 divided doses) was compared to GLB individually titrated to provide optimal glycaemic control in a sub-set of 231 type 2 diabetics, previously treated by diet and exercise alone, in this 12 month randomised, double-blind study. **Results:** RSG 4mg and 8mg/day reduced both FPG and HbA1c at month 12 compared with baseline (both p<0.0001). RSG 8mg/day was more effective than optimally titrated GLB at producing sustained reductions in FPG over 12 months (p=0.001). RSG 8mg/day and GLB reduced HbA1c by similar amounts. More patients in the RSG 8mg/day group (54%) than in the GLB group (36%) had a ≥1.7mmol/L (30mg/dL) decrease in FPG. More patients in the RSG 8mg/day group (74%) than in the GLB group (49%) achieved a target FPG of <7.8mmol/L (140mg/dL). The results are shown in the table below. RSG was safe and well tolerated and did not cause hypoglycaemia (<1% vs 16% for GLB). **Conclusions:** RSG is more effective when used as first-line therapy than optimally titrated GLB in lowering FPG after 12 months in patients with type 2 diabetes.

	GLB	RSG 4mg/day	RSG 8mg/day
FPG (mmol/L)	**(n=77)**	**(n=82)**	**(n=72)**
Mean baseline	9.2	9.4	9.5
Mean Δ from baseline ± SD	-1.2 ± 1.89	-1.0 ± 1.92	-2.3 ± 2.10
p-value vs GLB		0.217	0.001
% with reduction ≥1.7mmol/L (30mg/dL)	36	35	54
% attaining <7.8mmol/L (140mg/dL)	49	45	74
HbA1c (%)			
Mean baseline	7.7	7.8	7.9
Mean Δ from baseline ± SD	-0.86 ± 0.86	-0.58 ± 0.98	-0.93 ± 1.23
p-value vs GLB		0.017	0.956

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ROSIGLITAZONE IS SUPERIOR TO GLYBURIDE IN REDUCING FASTING PLASMA GLUCOSE IN TYPE 2 DIABETIC PATIENTS

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Aims: To evaluate the efficacy of rosiglitazone (RSG) monotherapy as compared with optimally titrated glyburide (GLB, also known as glibenclamide) **Materials and Methods:** Twelve-month, randomized, double-blind study of 587 type 2 diabetic (T2D) patients receiving RSG (4 or 8mg/d, in 2 divided doses) or GLB (optimal titration). At recruitment, patients were being treated either by diet alone or by oral therapy, which was withdrawn 6 weeks before randomization. **Results:** RSG 8mg/d was more effective than optimally titrated GLB at producing sustained reductions in fasting plasma glucose (FPG) over 12 months. According to predefined criteria, RSG 8mg/d was statistically equivalent to GLB in its ability to reduce HbA1c. The overall incidence of adverse events was similar in all 3 treatment groups. Symptoms of hypoglycemia were reported more frequently with GLB (12%) than with RSG at 4mg/d (<1%) or 8mg/day (<2%). No patients had transaminase levels >3x upper limit of normal. **Conclusions:** RSG offers a viable alternative first-line oral treatment to GLB.

	GLB (titrated) (n=203)	RSG 4mg/day (n=195)	RSG 8mg/day (n=189)
FPG (mmol/L)			
Mean baseline	10.54	10.5	10.9
Mean Δ from baseline ± SD	-1.7±2.5	-1.4±2.3	-2.3±2.5
P value (RSG vs. GLB)	-	0.210	0.033
% with reduction ≥1.7mmol/L	48%	39%	58%
% achieving <7.8mmol/L	37%	36%	51%
HbA1c (%)			
Mean baseline	8.15	8.07	8.21
Mean Δ from baseline ± SD	-0.72±1.00	-0.27±1.04	-0.53±1.31

PS 69
New Therapies

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INTRA-INDIVIDUAL VARIABILITY OF THE METABOLIC EFFECT OF INHALED INSULIN AND IMPACT OF AN ABSORPTION ENHANCER

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Aims: To study the metabolic effect and its variability elicited by inhalation of microcrystalline solid insulin (87.2 U) combined with an absorption enhancer on three different study days in comparison to that of regular insulin (10.2 U) injected s.c. and to that of regular insulin (5.5 U) given i.v. **Material and Methods:** In random order 11 healthy volunteers received the three different insulin administrations on five study days. The metabolic effect of the insulin's was assessed as the glucose infusion rate needed to keep glycaemia constant during euglycaemic glucose clamps. **Results:** After inhalation of insulin the onset of action was substantially more rapid than after s.c. insulin injection and maximal action was reached earlier (86±47 vs. 182±53 min; $p<0.001$). The maximal glucose infusion rate after inhalation of insulin was comparable to that after subcutaneous insulin injection (9.2±2.6 vs. 8.8±2.8 mg/kg/min; n.s.). The glucose infusion rates in the first 120 min after inhalation were significantly greater than after s.c. insulin injection (area under the glucose infusion rate curve: 0.88±0.25 vs. 0.59±0.20 g/kg/120 min; $p<0.001$). However, the total metabolic effect after inhalation was comparable (2.50±0.76 vs. 2.56±0.69 g/kg/480 min; N.S.). The relative biopotency of inhaled insulin calculated with regard to the data from the s.c. insulin application was 12.0±3.5 %. Within the first 120 min after insulin application the relative biopotency was 18.5±3.7 %. The intra-individual variability of the metabolic response induced by insulin inhalation was 14±9 % for the maximal glucose infusion rate, 15±10 % for the time to the maximal effect and 16±12 % for the total amount of glucose infused. **Conclusions:** Addition of an absorption enhancer led to a considerably more pronounced metabolic effect in the first hours after insulin inhalation in comparison to inhalation of pure insulin. The variability of the metabolic effect elicited by inhaled insulin is comparable to that seen after s.c. injection of regular insulin.

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EFFECTS OF *AVERRHOEA BILIMBI* LEAF EXTRACT ON BLOOD GLUCOSE AND LIPIDS IN STREPTOZOTOCIN-DIABETIC SPRAGUE DAWLEY RATS.

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Aim: Decoction of the leaves of the *Averrhoea bilimbi* Linn. (Oxalidaceae) is taken in Indonesia as antidiabetic medication. The present study was designed to investigate the hypoglycaemic and hypolipidaemic activities of an ethanolic leaf extract of *A. bilimbi* (ABe) in streptozotocin (STZ) diabetic male SD rats. **Materials and Methods:** Male SD rats aged 10 weeks (200-250g) were made diabetic by injecting a single dose of 60mg of STZ/kg body weight intraperitoneally after a 24 hours fast. The leaves of *A. bilimbi* were exhaustively extracted with 80% ethanol, concentrated at 40°C using a rotavapor and freeze-dried to obtain a yellowish green powder. The optimal hypoglycaemic dose (125mg/kg bw) was determined by performing OGTT in both normal and diabetic rats. To investigate the effect of long term administration of ABe, diabetic animals (n=5-6) were treated with vehicle (distilled water), ABe (125mg/kg bw) or metformin (500mg/kg bw), twice a day for 14 days. **Results:** Similar to metformin, ABe lowered both the blood glucose and triglyceride concentrations by 49.8% (15.1 ± 2.7 vs 30.1± 4.7 mmol/l, $p<0.01$) and 130% (0.5± 0.1 vs 1.8 ± 0.5 mmol/l, $p<0.05$) respectively when compared with vehicle. ABe also increased the HDL-C concentrations by 60% compared with vehicle (0.8 ± 0.01 vs 0.5 ± 0.03 mmol/l, $p<0.05$). Hence ABe increased the anti-atherogenic index ($p<0.001$) and HDL-C:TC ratio ($p<0.01$) significantly. However, like in the metformin treated group, there was no change in total cholesterol and LDL-cholesterol concentrations whereas the lipid peroxidation level was significantly reduced in the kidneys of the ABe-treated rats ($p<0.01$). **Conclusion:** The ABe shows hypoglycaemic, hypotriglyceridaemic, anti-lipid peroxidative and anti-atherogenic properties. Further studies are in progress to purify and isolate the active principle(s) in ABe.

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PRAMLINTIDE THERAPY IN ADDITION TO INSULIN IN TYPE 1 DIABETES: EFFECT ON METABOLIC CONTROL AFTER 6 MONTHS.

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Improving glycemic control in type 1 diabetes using intensive insulin therapy is often associated with increased hypoglycemia and weight gain. Pramlintide (PRAM) is a synthetic analog of the human β-cell hormone amylin. Amylin is deficient in type 1 diabetes. To evaluate the effects of concomitant pramlintide/insulin therapy on metabolic control in people with type 1 diabetes, this 26-week, multicenter, double-blind, placebo-controlled study was conducted in Europe and Canada. 586 subjects were treated subcutaneously with insulin and either PRAM 60 µg TID, 90 µg BID, 90 µg TID or placebo TID for 26 weeks. Mean age was 38 yrs, mean duration of diabetes was 16 yrs and mean baseline HbA1c was 9.0%. Using an intent-to-treat analysis, 26 week changes in HbA1c were +0.1 for placebo compared to –0.2% ($p=0.007$), –0.1% ($p=0.048$), and –0.1% ($p=0.105$) for the 60 TID, 90 BID, and 90 TID groups respectively. A subset of subjects (those observing a reduction in HbA1c of ≥0.5% at 4 weeks of treatment) achieved greater decreases in HbA1c throughout the duration of the trial. Only 24.8% of subjects receiving insulin alone had a reduction in HbA_{1c} of ≥0.5% at 4 weeks compared to 41-45% of subjects receiving PRAM ($p<0.005$). At 26 weeks, those PRAM subjects had a mean ± sem reduction in HbA1c of 0.6% ± 0.27%, 0.4% ± 0.09%, and 0.5% ± 0.11% for the 60 TID, 90 BID, and 90 TID groups respectively. The most common drug-related side effect was mild nausea, which generally dissipated during the initial 8 weeks of therapy. The annual event rate for severe hypoglycemia was slightly higher than placebo for the 90 µg dose groups and was similar to placebo for the 60 µg TID group. All groups receiving pramlintide experienced weight loss compared to weight gain in subjects receiving only insulin. The mean change in body weight at 26 weeks was –1.6 ± 0.27 kg, –0.7 ± 0.30 kg, and –1.6 ± 0.27 kg for the 60 TID, 90 BID, and 90 TID groups respectively compared to +0.3 ± 0.38 kg for the insulin alone group. Thus, compared with placebo, the addition of pramlintide to existing insulin therapy resulted in clinically relevant improvements in glucose and weight control in subjects with type 1 diabetes.

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PRAMLINTIDE THERAPY IN ADDITION TO INSULIN IN TYPE 2 DIABETES: EFFECT ON METABOLIC CONTROL AFTER 6 MONTHS.

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Improvements in glycemic control using intensive insulin therapy are often associated with weight gain in people with type 2 diabetes. Pramlintide (PRAM) is a synthetic analog of the human β-cell hormone amylin, which is deficient in type 2 diabetes relevant to the prevailing plasma glucose concentrations. To evaluate the effects of concomitant pramlintide/insulin therapy on metabolic control in people with type 2 diabetes, a 26-week, multicenter, double-blind, placebo-controlled study was conducted in Europe and Canada. 499 subjects were treated subcutaneously with insulin and either PRAM 90 µg BID, 90 µg TID, 120 µg BID or placebo TID for 26 weeks. Mean age was 58 yrs, mean duration of diabetes was 13.5 yrs and mean baseline HbA1c was 9.4%. Using an intent-to-treat analysis, 26 week changes in HbA1c were –0.1 for placebo compared to –0.3% ($p=0.068$), –0.4% ($p=0.079$), and –0.4% ($p=0.028$) for the 90 BID, 90 TID, and 120 BID groups respectively. A subset of subjects (those observing a reduction in HbA1c of ≥0.5% at 4 weeks of treatment) achieved greater decreases in HbA1c throughout the duration of the trial. Only 25.2% of subjects receiving insulin alone (placebo) had a reduction in HbA1c of ≥0.5% at 4 weeks compared to 47-50% of subjects receiving PRAM ($p<0.001$). At 26 weeks, PRAM subjects meeting this criteria had a mean ± sem reduction in HbA1c of 0.8% ± 0.1%, 0.7% ± 0.1%, and 0.8% ± 0.1% for the 90 BID, 90 TID, and 120 BID groups respectively. The most common drug-related adverse event was mild nausea, which generally dissipated during the initial 8 weeks of therapy. There were no differences in the annual event rate for severe hypoglycemia among the groups. All groups receiving pramlintide experienced weight loss compared to weight gain in subjects receiving only insulin. The mean change in body weight at 26 weeks was –0.8 ± 0.3 kg, –1.3 ± 0.3 kg, and –1.4 ± 0.3 kg for the 90 BID, 90 TID, and 120 BID groups respectively compared to +0.1 ± 0.3 kg for the insulin alone group. In conclusion, compared with placebo, the addition of pramlintide to existing insulin therapy resulted in clinically relevant improvements in glucose and weight control in subjects with type 2 diabetes.

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NOVEL ANTIOXIDANT PHENSUCCINAL ATTENUATES THE DEVELOPMENT OF DITHIZONE–DIABETES IN RABBITS

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We have previously shown that the new succinate derivative Phensuccinal (Ph), a potent antioxidant, offers protection to pancreatic beta-cells against streptozotocin insult. **The aim** of the study was to assess whether Ph-treatment would correct the diabetogenic effects of dithizone (D). **Materials and methods:** Male chinchilla rabbits were given Ph per os (25 mg/kg) for a week before and a week after D-injection (35 mg/kg i.v.). Control diabetic (CD) and nondiabetic (C) groups were included in the study. Plasma samples were collected at day 7 after diabetes induction and analysed for glucose and insulin. Oxidative status of experimental animals was assessed by determination of plasma malonic dialdehyde (MDA), diene conjugates (DC) contents and catalase activity (CA). **Results:** The treatment with Ph significantly diminished D-induced elevation in blood glucose levels (13.8±1.7 vs CD: 24.0±2.5, $p<0.01$; C: 4.7±0.2 mmol/l) and increased insulin contents by 30 % ($p<0.05$) in comparison with control diabetic animals. Ph-administration suppressed oxidative stress reducing MDA contents (0.65±0.07 vs CD: 1.37±0.32, $p<0.01$; C: 0.53±0.07 µmol/ml), DC levels (0.30±0.03 vs CD: 0.41±0.02, $p<0.05$; C: 0.28±0.02 µmol/ml) and increasing CA 1,3-fold ($p<0.02$) compared to non-treated diabetic group. Histological examination of pancreas revealed a marked damage to the islet architectural pattern in diabetic control group and moderate morphological alterations in Ph-treated animals. **Conclusions:** The results suggests that Ph attenuates the development of D-induced diabetes in rabbits, possibly, due to its antioxidant and Zn-chelating properties.

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EFFECT OF A NATURAL COMPOUND ON GLUCOSE LEVEL, LIPID PROFILE AND TOTAL ANTIOXIDANT STATUS OF TYPE 1 DIABETIC RATS

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Aims: A compound (GC 07) isolated from *Gymnema sylvestre* extract a typical plant, used for the treatment of diabetes mellitus, has previously been shown to posses hypoglycemic effects in Type 1 diabetic rats. The compound has now been investigated for its timing of action during streptozotocin induced damage to rat islets. **Materials and Methods:** A single intraperitoneal injection of streptozotocin (65 mg/kg body weight) was given to 3-4 month old Long-Evans rats. Four groups comprising 6 rats in each group were then fed with GC 07 (50 mg/kg body weight) starting at 0(immediate), 3, 5, 7 days after the injection along with equal number of Control rats fed with 0.25% carboxy methyl cellulose (10 ml/kg body weight). Fasting blood samples were collected on the first day before stretozotocin injection and then day 3, 5 and 7 as appropriate for each group. In all groups final sampling was done after 7 days of feeding of the compound. Blood glucose was measured by glucose oxidase method and total antioxidant status (TAS) was assessed by a recently introduced colorimetric Kit. **Results:** GC 07 showed significant hypoglycemic effect in all the groups except the 0 day group.

Parameter	0 day		3rd day		5th day		7th day	
	Cont	Treat	Cont	Treat	Cont	Treat	Cont	Treat
Glucose (mmol/l) (n=6)	26.7±4.2	26.9±4.1	22.1±5.9	10.4±6.6 p<0.009	28.4±5.8	17.2±4.8 p<0.002	27.4±7.8	17.9±5.6 p<0.03
TAS (mmol/l) (n=6)	0.86±.05	0.90±.04	1.17±.31	1.06±.07	0.94±.20	1.08±.06	0.91±.06	0.91±.07

Cont= Control;Treat= Treated; p= Significance compared to Control

Conclusion: The results indicate that the compound is not probably able to prevent the damaging effect of streptpozotocin on pancreatic B cells but it may help in recovering the damaged cells. The effect of the compound on B cells does not seem to related to the total antioxidant status of the body. GC 07 merit further study regarding its mechanism of action.

PS 70
Prolonged Acting Insulins

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NEW PRINCIPLE FOR PROLONGING THE ABSORPTION OF HUMAN INSULIN SUSPENSION PREPARATIONS

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Aim: To investigate the influence on absorption of the suspension preparations, human NPH and Semilente, injected subcutaneously in a given dose, by increasing their insulin concentration five fold. **Materials and Methods:** In each of 14 experiments, 10 IU (20 µl) human NPH U500, labelled with 125-I-insulin, and 10 IU (100µl) human NPH U100, labelled with 125-I-insulin, were injected contralaterally to a depth of 5 mm in the neck of a pig, followed by monitoring residual radioactivity over the injection sites at 0, 2, 4, 24 and 48 h after injection. In a second study, labelled human Semilente U500 and U100 were injected and examined correspondingly in 4 experiments. **Results:** For NPH, U500 *vs* U100, the time until 75%, 50% and 25% residual radioactivity, respectively, was (mean hour± SEM, with p-values of a paired t-test, n=14): T75%: 5.0±0.5 h *vs* 3.8±0.3 h (p=0.007), T50%: 12.2±0.9 h *vs* 9.0±0.6 h (p=0.003) and T25%: 24.2±1.2 h *vs* 17.9±1.2 h (p=0.001). In the second study (n=4), the corresponding values for Semilente, U500 *vs* U100, were: T75%: 2.7±0.7 h *vs* 1.2±0.1 h (p=0.11), T50%: 5.3±1.0 h *vs* 2.8±0.3 h (p=0.07) and T25%: 10.1±1.5 h *vs* 5.8±1.0 h (p=0.05). **Conclusions:** The absorption of human NPH and Semilente is substantially prolonged by increasing their insulin concentration five fold. NPH U500, in a specially adapted insulin pen, appears to be more appropriate for a single injection a day in basal insulin therapy than NPH U100.

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PHARMACOKINETICS AND DYNAMICS OF S.C. INJECTION OF THE LONG-ACTING INSULIN GLARGINE (HOE 901) IN T1DM.

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To establish the pharmacokinetics/dynamics of the long-acting insulin glargine (HOE 901), 20 T1DM patients were studied in a two-way cross-over clamp design in the post-absorptive state (plasma glucose, PG, regulated by i.v. insulin at 7.2 mmol/l) after s.c. injection of 0.3 U/Kg of HOE 901 or NPH insulin. After s.c. injection, i.v. insulin was tapered and glucose infused to clamp PG at 7.2 mmol/l up to 24 hours, but the study was discontinued whenever PG was >11.1 mmol/l. Onset of action (time of reduction of i.v. insulin by 50%) was later with HOE 901 vs NPH (medians: 1.11 vs 0.71 h, $p<0.08$) and duration of action (end of action, i.e. PG>8.3 mmol/l, minus onset of action) longer (medians: 22.8 vs 13.8 h, ($p<0.002$). Glucose infusion rate (GIR, mg/kg/min), PG (mg/dl) and plasma free insulin (FIRI, mU/ml) are reported below (time indicates hours after s.c. injection, mean ± SEM).

Time (h)		0	6	12	18	24
FIRI	HOE	12±4	10±2	11±2	10±2	8.8±3
	NPH	12±5	19±9	13±3	9±4	discontinued
GIR	HOE	0	0.9±0.7	0.7±0.7	0.7±0.8	0.6±0.7
	NPH	0	3.2±2.7	1±1.1	0	discontinued
PG	HOE	129±2	129±2	134±14	140±24	140±23
	NPH	130±1	137±1	140±15	178±26	discontinued

In contrast to NPH peak (FIRI at 4 h of 22±10 mU/ml, GIR at 5h of 3.3±2.6 mg/kg/min), HOE 901 had a peakless, long lasting concentration/action profile closely mimicking a "square-wave" shape. Intersubject coefficient of variation of area under FIRI curve was lower with HOE 901 vs NPH (19 vs 31%). Conclusions: HOE 901 has more prolonged and peakless activity, and more reproducible pharmacokinetics than NPH.

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EFFICACY AND SAFETY OF INSULIN GLARGINE IN TYPE 1 DIABETES: A 28 WEEK RANDOMIZED, NPH-INSULIN CONTROLLED TRIAL.

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Aims: Insulin glargine (21^{A}-Gly-30^{B}a-L-Arg-30^{B}b-L-Arg-human insulin) is a biosynthetic insulin analog with prolonged biological action compared to NPH. This multicenter, randomized, parallel study compares insulin glargine with NPH in type 1 DM subjects previously treated with multiple daily injections (MDI) of NPH and regular insulin.

Materials and Methods: 534 well-controlled subjects (mean age 38.5 yrs, mean DM duration 17.4 yrs, mean glycohemoglobin (GhB) 7.7%, mean FPG 11.8 mmol/L) were treated for up to 28 weeks in 49 centers and randomized to receive pre-meal regular insulin and either bedtime insulin glargine, or bedtime/BID NPH.

Results: Given good glycemic control at baseline, only a small decrease in GhB was noted in both Insulin glargine (-0.16%) and NPH (-0.21%). Better glycemic control was achieved by insulin glargine with a reduction in FPG (-1.67mmol/L vs. -0.33 mmol/L, p=0.01) and a trend toward more subjects achieving ADA goals of therapy (66.4% vs. 58.9%). In the DCCT, the limitation of intensive diabetes therapy was the occurrence of hypoglycemia, particularly during sleep. After a one month titration phase, the frequency and severity of reactions were: *p <0.05

	Hypoglycemia < 2.0 mmol/L	Severe Hypoglycemia	Nocturnal Hypoglycemia < 2.0 mmol/L
Insulin Glargine	39.9% 200.5 episodes/ 100pt-yrs	6.6% 24.6 episodes/ 100pt-yrs	18.2% 65.1 episodes/ 100 pt-yrs
NPH	49.2% * 345.4 episodes/ 100pt-yrs	8.6% 37.6 episodes/ 100pt-yrs	27.1% * 101.2 episodes/ 100pt-yrs

Conclusions: Insulin glargine achieves better glycemic control with fewer episodes of hypoglycemia compared to either once or twice daily NPH when used with regular insulin in an MDI regimen in patients with type 1 diabetes.

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CHARACTERIZATION OF GLUCOSE TURNOVER OF INSULIN GLARGINE IN COMPARISON WITH REGULAR HUMAN INSULIN IN HEALTHY MALE SUBJECTS.

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Aims: The long-acting human insulin analogue, glargine (21^{A}-Gly-30^{B}a-L-Arg-30^{B}b-L-Arg-human insulin) has a carboxy terminal elongation of the β chain by 2 arginines and replacement of asparagine in position A21 by glycine. We utilized the hyperinsu-linemic euglycemic clamp to assess possible pharmacokinetic and pharmacodynamic differences between insulin glargine (IG) and regular human insulin (RHI). **Materials and Methods:** The study design was a single-dose, double-blind, randomized, cross-over trial involving lean, non-diabetic men (n=12). Subjects received a primed, continuous intravenous infusion of IG or RHI at a rate of 40 mU/m²/min for 240 mins. Euglycemia was maintained at 90 mg/dl using a simultaneous variable intravenous infusion of 20% dextrose containing D-[3-^{3}H]-glucose. Following insulin infusion, euglycemia was sustained for an additional 180 minutes to determine the rate and duration of the insulin effect. **Results:** There were no significant pharmacodynamic differences in the mean (± SEM) for A_{50} HGO (i.e. the time in minutes required for 50% suppression of hepatic glucose output following insulin infusion) at 73.0± 23.1 vs. 56.6 ± 19.6, p=0.06, the maximal rates of glucose disposal (Rdmax) at 10.1 ± 0.8 vs. 9.9 ± 0.9 mg/kg/min (p>0.9), incremental activation (A_{50}) 32.4 ± 4.6 vs 42.1± 9.5 min, p>0.3 or deactivation (D_{50}) 62.8 ± 5.3 vs. 57.3 ± 3.6, p>0.4 for RHI or IG, respectively. **Conclusions:** The results demonstrated no significant difference in activation or deactivation between intravenous IG and RHI. Thus, kinetic differences between IG and RHI when given subcutaneously are completely due to differences in absorption kinetics. The unique amino acid sequence of IG is thought to be respon-sible for the prolonged kinetics. Thus, IG may truly be a long-acting insulin which may mimic the body's own basal insulin secretion when administered subcutaneously.

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TIME-ACTION PROFILE OF THE LONG-ACTING INSULIN ANALOGUE HOE901

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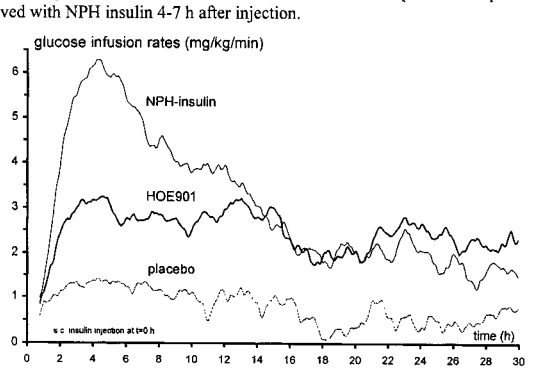
Aims: To study the pharmacodynamic and pharmacokinetic properties of the subcutaneously injected long-acting insulin analogue HOE901 (30 µg/ml zinc) in comparison to NPH insulin and placebo. **Material and Methods:** In this single-center double-blind euglycemic glucose clamp study 15 healthy male volunteers (age, 27±4 years; BMI, 22.3±1.8 kg/m²) received single subcutaneous injections of 0.4 U/kg weight of either HOE901, NPH insulin or placebo on three study days in randomized order. Glucose infusion rates (GIR) were registered over a 30-h period after administration. **Results:** The time-action profile of HOE901 did not show the pronounced peak in metabolic activity seen with NPH insulin (figure; GIR_{max} 5.3±1.1 vs. 7.7±1.3 mg/kg/min; p<0.05). The metabolic effect (measured as areas under the curves (AUC)) was lower with HOE901 compared to NPH insulin in the first 4 h after injection ($AUC_{0\text{-}4\ h}$ 1.02±0.34 vs. 1.48±0.34 g/kg*4 h; p<0.01), but was comparable over the total study duration ($AUC_{0\text{-}30\ h}$ 7.93±1.82 vs. 9.24±1.29 g/kg*30 h; ns). **Conclusions:** This study shows that the time-action profile of the soluble long-acting insulin analogue HOE901 is smoother and does not show the pronounced peak action observed with NPH insulin 4-7 h after injection.



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A COMPARISON OF THE PHARMACODYNAMICS (GLUCOSE LOWERING EFFECT) OF INTRAVENOUS HOE 901 AND REGULAR INSULIN USING THE EUGLYCAEMIC CLAMP TECHNIQUE.

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Background: Modification of the human insulin molecule by recombinant techniques led to the production of insulin glargine (HOE 901), a novel new long-acting insulin analogue, to be used as a once daily basal insulin.

Aim: The aim of this study was to compare the pharmacodynamics (glucose lowering effect) of equimolar insulin glargine (HOE 901) and regular semi-synthetic human insulin, administered as a short intravenous infusion to healthy volunteers, using the euglycaemic clamp technique.

Materials and methods: This was a single-dose, double-blind, randomized, two-way crossover study with a wash-out period of 7 days between treatments. Twenty healthy volunteers (5 female, 15 male), aged between 18 and 45 years received HOE 901 and regular insulin (0.1 IU/kg) as a short (30 minute) constant intravenous infusion, after which they were clamped for 6 hours at their own individual fasting blood glucose concentrations.

Results: The mean values of the primary variable $AUC_{(0\text{-}6)}$ of the glucose infusion rate (GIR) were 663.9 and 734.9 mg/kg for HOE 901 and H-insulin respectively. The 90% confidence interval for the mean ratio "HOE 901/H-insulin" was 84.6-96.7% (point estimate 90.3%) which was within the predefined conventional equivalence range of 80-125%. The intra-individual CV was 12% (as calculated from the mean square of error in the ANOVA table). Secondary variables of GIR, including GIR_{max}, also fell within the conventional equivalence range.

Conclusion: Equimolar insulin glargine (HOE 901) and regular insulin, administered by short intravenous infusion, were equivalent with respect to their glucose lowering effect in healthy volunteers, using the euglycaemic clamp technique.

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AN ASSESSMENT OF THE VARIABILITY IN THE PHARMACODYNAMICS (GLUCOSE LOWERING EFFECT) OF HOE901 COMPARED TO NPH AND ULTRALENTE HUMAN INSULINS USING THE EUGLYCAEMIC CLAMP TECHNIQUE

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Background: The lack of reproducibility in the glucose lowering effect of s.c. insulin preparations (more pronounced in the longer-acting insulins such as NPH and Ultralente), is a serious problem for most IDDM patients. HOE901 (glargine insulin), a new long-acting basal insulin, with a delayed and prolonged absorption from the injection site, might be less variable in its glucose lowering effect.

Aim: The aim of this study was to compare the intra-subject variability in glucose lowering effect of s.c. HOE901, NPH and Ultralente insulin in healthy volunteers using the euglycaemic clamp technique.

Materials and methods: This was a single-dose, double-blind, randomized, parallel (3 groups of 12) replicate design. Thirty-six male subjects, aged between 18 and 45 years, received 2 consecutive s.c. injections (0.4 IU/kg) of one of the 3 study treatments (HOE901, NPH, Ultralente), with a wash-out period of 7 days between treatments. Euglycaemic glucose clamps were performed for up to 24 hours after drug administration at the subject's own individual fasting blood glucose concentrations.

Results: The mean values and intra-individual CV (in brackets) of the main pharmacodynamic variable, AUC (mg/kg) of the glucose infusion rate (GIR), for the 2 visits of the 3 treatments were as follows:

	HOE901	NPH	Ultralente
AUC (0-24h)	2558; 2987 **(32%)**	3117; 2847 **(19%)**	2593; 2025 **(38%)**
AUC (12-24h)	1264; 1217 **(23%)**	1019; 1101 **(29%)**	1071; 1213 **(55%)**

Conclusion: The intra-subject variability of the pharmacodynamics (glucose lowering effect) during the entire 24-hour clamp period was the lowest with NPH, followed by HOE901 and Ultralente. However, during the latter part of the time-action profile (from 12 hours after injection onwards), HOE901 showed the lowest intra-subject variability. It is concluded that the reproducibility of the glucose lowering effect of HOE901 is comparable to that of NPH, but better than that of Ultralente.

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INSULIN GLARGINE (HOE 901) LOWERS FASTING BLOOD GLUCOSE IN CHILDREN WITH TYPE I DIABETES MELLITUS WITHOUT INCREASING THE RISK OF HYPOGLYCAEMIA.

E Schoenle on behalf of the HOE 901/3003 study group

Aim: The peakless, prolonged time-action profile of insulin glargine is particularly important for a basal insulin in type I diabetic children for whom the risks of nocturnal hypoglycaemia and high fasting blood glucose are especially great. This study compared insulin glargine with human NPH insulin in children with type 1 diabetes mellitus. **Methods:** This was a multicenter, open, randomised study with a parallel-group design. The basal insulin dosing regimen for subjects receiving insulin glargine was once daily (at bedtime) regardless of their prior regimen, while the regimen for subjects receiving NPH insulin was either once (at bedtime) or twice daily (in the morning and at bedtime), based on their regimen prior to the treatment phase. The daily insulin dose was adjusted to maintain FBG between 4.4 and 8.8 mmol/L while avoiding hypoglycaemia. Human regular insulin was injected before meals according to common practice. **Results:** 349 subjects with type 1 diabetes mellitus between 5 and 16 years of age were treated for 6 months, 174 with insulin glargine and 175 with NPH. The change in GHb was comparable in both treatment groups, but children treated with insulin glargine had a greater mean decrease in FBG (-1.27 mmol/L) than NPH subjects (-0.70 mmol/L). This difference was statistically significant (p=0.0315). While the overall frequency of hypoglycemia was similar in both treatment groups (Insulin glargine: 79.3%, NPH: 78.9%), insulin glargine treated children reported less frequent severe (22.4% vs. 28.6%), nocturnal (48.3% vs. 50.9%) and severe nocturnal (12.6% vs. 17.7%) hypoglycemia than NPH subjects. Insulin glargine was well tolerated and had a safety profile similar to human insulin, including injection site reactions and human insulin and *E. coli* antibodies. **Conclusion:** In children, a single, evening subcutaneous dose of insulin glargine provides lower FBG values than NPH, with a diminished risk of nocturnal and severe hypoglycaemia.

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HOE 901 (INSULIN GLARGINE) IMPROVES SATISFACTION WITH INTENSIFIED INSULIN TREATMENT FOR TYPE 1 DIABETES

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Aim: Insulin glargine, a new long-acting insulin, provides constant, peakless insulin release and is designed for once daily administration. Satisfaction with treatment (Diabetes Treatment Satisfaction Questionnaire (DTSQ)) and psychological well-being (Well-being Questionnaire (W-BQ)) were assessed in 9 European countries in a phase III multicentre randomised controlled open clinical trial comparing the effects of insulin glargine and NPH human basal insulin. **Methods:** The questionnaires, were self-completed at baseline, and at least once at weeks 8, 20 or 28 by the 517 patients (287 men and 230 women; mean age 39.7±12.1 yrs; mean duration of diabetes 16.0± 10.7 yrs; mean GHb at baseline 7.9±1.2 %). Baseline scores of the DTSQ (mean 27.96 ± 5.65; maximum possible score = 36) and of the W-BQ (mean 49.88 ± 9.13; maximum possible score = 66) were high in this population. Analysis of covariance was performed on the change from baseline scores, using treatment and pooled site as main effects with baseline scores as covariate. **Results:** Treatment satisfaction improved during treatment with insulin glargine at all time points and at endpoint (last on-treatment assessment) whereas it deteriorated slightly with NPH insulin. These differences were significant throughout the study (change from baseline at end point +1.27 vs −0.56; p = 0.0001). Outcomes were better with insulin glargine for both Perceived Frequency of Hyperglycaemia and Perceived Frequency of Hypoglycaemia with statistically significant differences at week 28 and at endpoint for hyperglycaemia (p = 0.037 and 0.038) and at week 20 (p = 0.002) for hypoglycaemia. Glycaemic control, as measured by GHb, was essentially unchanged in both treatment groups. There was no difference in psychological well-being between the treatment groups. Mean W-BQ scores in these patients increased during the study in both groups (pvalue). Analysis of the W-BQ subscales shows that the observed effects on general well-being are attributable to effects on all subscale scores. **Conclusion:** The psychological outcomes observed in this study include treatment-independent improvements in general well-being. More substantial effects were seen on treatment satisfaction which was statistically significantly improved with insulin glargine, together with a decrease in perceived frequency of hyperglycaemia and hypoglycaemia compared with NPH.

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PHARMACOKINETICS AND PHARMACODYNAMICS OF FATTY ACID-ACYLATED HUMAN INSULINS IN CONSCIOUS SWINE.

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Aims: Fatty acyl insulins bind to plasma albumin, extending their sojourn in the circulation and their action, and altering their kinetics and pharmacodynamics. The dose responses of [N^{ε}-myristoyl Lys(B29)]- and [N^{ε}-myristoyl Lys(B28)Pro(B29)]-human insulins (C14-HI and C14-lispro, resp.) with their intravenous infusion were therefore characterized in swine. **Materials and Methods:** 20h-fasted conscious pigs underwent 6h infusions of C14-HI, C14-lispro and regular human insulin (HI) at 5, 10, 20 and 60 pmol/kg-min (n=5 each) and of somatostatin at 0.3μg/kg-min, and a euglycemic clamp during and after the infusion. **Results:** (mean±sem) For the 5, 10 and 20 pmol/kg-min infusions, maximal total plasma IRI (nmol/L) were 0.19±0.03, 0.47±0.04, 0.71±0.07 for HI; 2.6±0.5, 5.0±0.4, 7.0±0.6 for C14-HI & 3.3±0.2, 6.9±0.8, 13.5±1.3 for C14-lispro. Max. glucose infusion rates (mg/kg-min) were 21.8±2.5, 28.4±2.3, 36.0±4.1 for HI; 13.0±2.3, 21.5±2.1, 29.5±2.0 for C14-HI and 14.1±2.5, 16.1±3.6, 29.6±2.7 for C14-lispro. Areas under the IRI response curves (μmol-min/L) were: 0.070±0.013,0.180±0.009, 0.259±0.038 for HI; 0.93±0.16, 1.82±0.16, 2.62±0.21 for C14-HI and 1.3±0.1, 2.65±0.35, 4.97±0.46 for C14-lispro with total glucose requirements (g/kg), respectively, of 6.5±0.7, 9.2±1.0 & 1 3.1±1.6 for HI; 4.8±1.0, 8.5±1.0 and 12.2±1.2 for C14-HI and 5.0±0.8, 7.2±1.5 and 14.0±2.0 for C14-lispro. The relationships between insulin area and glucose infused were highly linear for the two acylated insulins with a lower slope for C14-lispro ($p<0.05$). Saturating doses of these insulins (60 nmol/kg-min) increased maximal and cumulative total IRI, maintained maximal glucose infusion rates as for the 20 nmol/kg-min dose while increasing total glucose requirements to 21.9±2.1 and 20.8±2.0 g/kg for C14-HI and C14-lispro, resp. and increasing the time at which glucose infusion was half-maximal from 510 to 900min for C14-HI and 560 to 950min for C14-lispro.**Conclusions:** Over the therapeutic range, and viewed from the perspective of total circulating IRI, C14-HI and C14-lispro demonstrate the behavior of a slowly-cleared, lower-potency analog and a linear relationship between the IRI excursion and glucose requirements. Saturating doses are nonlinear with the dose effect expressed only as an extension of action.

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DETERMINATION OF *IN VITRO* PLASMA PROTEIN BINDING OF INSULIN ASPART AND INSULIN DETEMIR BY EQUILIBRIUM DIALYSIS

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Aims: Insulin Aspart (IAsp) and Insulin Detemir (IDet) are two insulin derivatives in development for the treatment of diabetes mellitus. Ultrafiltration and equilibrium dialysis are routine *in vitro* techniques for the determination of plasma protein binding. For IAsp and IDet, however, it is impossible to perform *in vitro* studies in a protein-free environment, due to the adherence of the peptides to the container and membrane surfaces. To overcome this obstacle, a step-wise equilibrium dialysis was devised. **Materials and methods**: The dialysis was performed as a sequence of step-wise, repeated experiments, involving the presence of peptide on both the donor and receptor side of the dialysis membrane. Each step was initiated at the concentration ratio corresponding to the final ratio determined in the previous experiment and each experiment was started at equilibrium between solution and container surfaces. Plasma from healthy human volunteers with drug levels at, above and below the expected therapeutic concentration, was used for determination of protein binding. Plasma was placed into a dialysis sack (MW cut-off: 25 kDa) and deposited in a buffered reservoir of the peptide (^{125}I-labelled at position 14-Tyrosine of the insulin A chain) at the expected free concentration in plasma. The plasma samples were then incubated at 37°C under gentle rotation for at least 20 h. Aliquots were taken from both sides of the membrane and counted for radioactivity. For IDet a pre-equilibrium of 90% was prepared by establishing a concentration ratio of ten between the plasma in the sack and the outer solution (free fraction). For IAsp and HI this ratio was 1:1, imitating 0% binding. **Results:** The extent of binding was 5–10% for IAsp and HI and 99% for IDet, and for all insulins binding appeared to be constant over the entire concentration range studied. **Conclusions:** The study shows that this step-wise equilibrium technique can be used to determine *in vitro* plasma protein binding for substances which exhibit strong adhesive and/or slow diffusion properties.

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AGE-DEPENDENT PHARMACOKINETICS OF HUMAN INSULIN AND INSULIN ASPART IN YOUNG PATIENTS WITH TYPE 1 DIABETES?

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Aims: The pharmacokinetics of the rapid-acting, human insulin analogue insulin aspart (IAsp), were compared with those of soluble human insulin (HI) in children and adolescents with type 1 diabetes. **Materials and Methods:** The trial had a randomised, double-blind, two-period crossover design. On each of the two study days, 9 children (aged 6–12 years) and 9 adolescents (aged 13–17 years) received a single subcutaneous dose (0.15 U/kg) of either IAsp or HI, administered in the abdominal wall 5 min before a breakfast. Plasma insulin and glucose concentrations were measured at intervals during the following 5 h. **Results:** The maximum serum insulin concentration was 147 ± 53.5 mU/l for IAsp and 70.3 ± 32.1 mU/l for HI (mean ± SD), p<0.001) and occurred earlier for IAsp; serum insulin t_{max} for IAsp was 40.0 min (IQ range 20–70 min) vs. 75.0 min (IQ range 40–180 min) for HI ($p < 0.001$). This resulted in a significant difference ($p < 0.05$) between IAsp and HI C_{max} (corrected for baseline glucose) of 7.64 ± 5.10 and 9.41 ± 4.38 respectively. For the two age groups, maximum insulin levels for IAsp were approximately twice those of HI (table). However for both insulin types (HI and IAsp) the C_{max} and $AUC_{0-5\,h}$ values were significantly higher ($p < 0.05$) in the adolescent (13–17 year) group.

	Children (mean ± SD)	Adolescents (mean ± SD)
IAsp - C_{max} (mU/l)	121 ± 39	173 ± 54
HI - C_{max} (mU/l)	59 ± 16	82 ± 40
IAsp - $AUC_{0-5\,h}$ (mU/l·h)	240 ± 106	438 ± 245
HI - $AUC_{0-5\,h}$ (mU/l·h)	179 ± 69	313 ± 186

Conclusions: IAsp displays a more rapid onset and shorter duration of action (insulin levels returned to baseline 5 h after dosing) compared with HI in children and adolescents, resulting in improved postprandial glucose control even when IAsp is administered immediately before a meal. The novel finding of a possible age effect on insulin absorption, suggested by the present results, requires further investigation.

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A PHARMACOKINETIC AND PHARMACODYNAMIC COMPARISON OF INSULIN ASPART IN HEALTHY SUBJECTS AND DIABETES PATIENTS

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Aims: Insulin aspart, a rapid-acting human insulin analogue, is currently being tested for meal-related treatment of diabetes mellitus. The pharmacokinetic and pharmacodynamic properties of insulin aspart were compared with those of soluble human insulin in healthy volunteers and in patients with Type 1 diabetes. **Materials and Methods:** Two trials were carried out in healthy volunteers (aged 19–50 yr, BMI <30.0 kg/m^2, HbA_{1c} <6.1% and fasting plasma glucose <6.0 mmol/l). In study 1, 19 healthy subjects received a subcutaneous dose of 0.06 U/kg of either insulin aspart or soluble human insulin under fasting conditions. Study 2 was similar in design except that the subcutaneous dose given to the 20 subjects was 0.10 U/kg. In the third study, 23 patients with Type 1 diabetes (aged 18–50 yr, BMI <30.0 kg/m^2, a meal-stimulated C-peptide <0.10 nmol/l, concomitant plasma glucose >7.0 mmol/l, no late diabetic complications) were given a subcutaneous dose of either insulin aspart or soluble human insulin (0.15 U/kg), before a breakfast. **Results:** The mean C_{max} of insulin aspart was approximately twice that of soluble human insulin in all three trials. When plotted against dose, a linear relationship was found between trials, and between healthy subjects (dose 0.06 or 0.10 U/kg) and those with diabetes (dose 0.15 U/kg). Regarding $AUC_{(ins)}$, the results of the individual trials indicated similar bioavailability of insulin aspart and soluble human insulin. Insulin t_{max} did not differ between healthy volunteers and diabetic patients (median 40 min). The pharmacokinetic-pharmacodynamic relationship as assessed by the time lapsed from C_{max} of insulin to C_{min} of glucose (healthy volunteers) or postprandial C_{max} of glucose (diabetic patients) was 30 min and 20 min respectively (median values). **Conclusions:** Patients with Type 1 diabetes and healthy volunteers exhibited similar pharmacokinetic and pharmacodynamic responses to subcutaneous administration of insulin aspart.

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PHARMACOKINETIC AND PHARMACODYNAMIC COMPARISON OF A PREMIX OF SOLUBLE AND PROTAMINE-RETARDED INSULIN ASPART

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Aims: The serum insulin profiles after a single injection of a 30/70 premix of the rapid-acting insulin analogue, insulin aspart and protamine-retarded insulin aspart (Biphasic Insulin Aspart 30 [BIAsp30]) were compared with a similar premix of human insulin (Biphasic Human Insulin 30 [BHI30]). **Materials and Methods:** In this randomised, double-blind, two-period crossover study, 24 healthy male subjects received a single subcutaneous dose of either BIAsp30 or BHI30 (0.2 U/kg) on two study days (4–10 days apart), following an overnight fast. Blood samples were taken for determination of serum insulin and glucose concentrations at various intervals after dosing. End-points were chosen to represent the soluble fraction of the insulin (early part of the profile) and the protamine-retarded fraction (later part of the profile). **Results:** The primary end-point, the area under the insulin concentration–time curve from 0 to 90 min after dosing was significantly larger for BIAsp30 than for BHI30 (1403 ± 372 versus 752 ± 191 mU/l h [mean ± SD]; $p < 0.0001$). The time to maximum serum insulin concentration was approximately half that for BHI30 (60.0 [45.0–70.0] versus 110.0 [90.0–180.0] min [median, interquartile range]; $p < 0.0001$) and the maximum insulin concentration was higher following BIAsp30 than after BHI30 (23.4 ± 5.3 versus 15.5 ± 3.7 mU/l [mean ± SD]; $p < 0.0001$). The serum glucose profiles showed a significantly earlier onset of the glucose-lowering effect and a significantly lower glucose concentration following BIAsp30 than after BHI30. **Conclusions:** The results indicate that the pharmacokinetic characteristics of insulin aspart, which are potentially important for postprandial glucose control, are preserved with insulin aspart used in a premixed formulation.

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NO DIFFERENCE IN HYPOGLYCAEMIC SYMPTOM THRESHOLD FOR INSULIN ASPART AND HUMAN INSULIN IN TYPE 1 DIABETES PATIENTS

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Aims: The aim of the study was to compare the blood glucose threshold for autonomic activation during hypoglycaemia induced by insulin aspart (IAsp) and soluble human insulin (HI). Counterregulatory, symptomatic and physiological responses were also compared and safety end-points were evaluated. **Materials and Methods**: The trial had a single-centre, double-blind, randomised, two-way cross-over design. On each of two study days (separated by 3–6 weeks), acute hypoglycaemia was induced in 17 patients (mean age 28.8±6.6 years, diabetes duration 2.4±1.3 years, BMI 23.6±1.8 kg/m^2, HbA_{1c} 6.69±0.67%) by infusion of either IAsp or HI (100 IU/ml at 2 mU/kg/min). **Results**: The mean blood glucose levels at the time of autonomic reaction, were not significantly different for IAsp, 1.88 (range 1.3–2.6) mmol/l and HI, 1.89 (range 1.3–2.7) mmol/l; (NS, estimated ratio = 0.994). Mean C_{max} for IAsp was 2.84 nmol/l and 3.69 nmol/l for HI (NS); t_{max} occurred at 53.8 min for IAsp and 53.4 for HI (NS). No statistically significant differences were found between IAsp and HI in any of the secondary endpoints, including the AUC, C_{max} and t_{max} for the counterregulatory hormones. Mean change in systolic blood pressure was 20.2 mmHg for IAsp and 15.2 mmHg for HI (NS); mean heart rate increased by 19 beats/min with IAsp and 22 beats/min with HI (NS); mean blood glucose slope was -0.081 (IAsp) vs. -0.083 (HI) mmol/l/min (NS). Mean hypoglycaemic scores were 24.4 for IAsp and 27.2 for HI (NS). Mean cognitive test results for IAsp and HI (Trail Test B) were 64.4 vs. 69.6 (NS) and (Digit test) 45.8 vs. 40.0 (NS) respectively. No serious adverse events were reported during the study. **Conclusions:** Analysis of pharmacodynamic and pharmacokinetic measures of insulin and counterregulatory hormonal responses indicates that IAsp generates the same symptoms and counterregulatory responses as induced by HI during hypoglycaemia. The safety profiles of the two insulins were also similar.

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SOLUBLE INSULIN AND THE RAPID -ACTING ANALOGUE INSULIN ASPART HAVE SIMILAR EFFECTS UPON VENTRICULAR REPOLARIZATION.

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Aims Abnormal cardiac repolarization develops during insulin induced hypoglycaemia, which in other conditions is associated with cardiac dysrhythmias. An increase in sudden death of young diabetic patients has been associated with nocturnal hypoglycaemia perhaps as a result of cardiac dysrhythmias. Since the use of rapid-acting insulin analogues has been suggested to reduce nocturnal hypoglycaemia we thought it important to establish their effect on these aspects of cardiac function. **Methods and Results** 17 healthy males (mean±SEM age 27.7±1.6yr, BMI 23.5±0.5kg/m^2) underwent identical hyperinsulinaemic hypoglycaemic clamps with blood glucose maintained at 5mM for 30 min followed by 30 min at 2.5mM. Subjects received either regular human insulin (HI) or the analogue, insulin aspart (IAsp) on 2 different occasions separated by 4-6 weeks. We made regular measurements of plasma potassium, counterregulatory hormones and 2 measures of cardiac repolarization, QT dispersion and QTc. During each study potassium decreased significantly but by a similar amount (Initial and final level, HI, 4.2±0.3 to 3.4±0.2mM,P<0.001, IAsp, 4.2±0.3, to 3.4±0.2mM, P<0.001). Serum adrenaline increased significantly and similarly after both insulins (Initial and final level, HI, 0.19±0.01, to 4.87±0.48nM,P<0.001, IAsp, 0.18±0.01 to 4.99± 0.48nM, P<0.001). There were similar significant increases from baseline in QTc and QT dispersion after 30min of hypoglycaemia induced by either HI (QTc, 79±6ms, P<0.001, dispersion 67±10ms, P<0.001), or IAsp (QTc, 86±8ms, P<0.001, dispersion, 53±8ms). There was no significant difference between treatments with regard to effect upon cardiac repolarization, potassium concentration and adrenaline levels. Clearance rates for the two insulins was similar (HI mean 1.24±0.12l/h/kg, IAsp mean 1.22±0.32l/h/kg).**Conclusions** We conclude that regular human insulin and insulin aspart have similar effects upon hypoglycaemia induced alterations in cardiac repolarization, presumably because the effects of both regular insulin and insulin aspart on the sympathoadrenal response and potassium concentration were the same.

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INSULIN ASPART – A MEALTIME ALTERNATIVE TO SOLUBLE HUMAN INSULIN IN TYPE 1 DIABETES.

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Aims: The safety and efficacy of insulin aspart (IAsp), a fast-acting insulin with a shorter duration of action than human insulin (HI), was assessed in a six-month, multi-center, randomized, open-label study. **Materials and Methods:** Eight hundred eighty two subjects with Type 1 diabetes, age ≥18 years, BMI ≤ 35 kg/m^2, and duration of diabetes ≥ 24 months, were studied. During a 4-week run-in period, subjects received a standard multiple injection treatment with HI and NPH insulin. During the treatment period IAsp (n = 596 subjects) was administered immediately before meals, while HI (n = 286 subjects) was administered 30 min before meals. Efficacy and safety were evaluated at 6 months. **Results:** At baseline, mean HbA_{1c} (% ± SD) was similar between treatment groups (7.9% ± 1.1 for IAsp, 8.0% ± 1.3 for HI). At 6 months of treatment, HbA_{1c} was lower for the IAsp group (7.8 % ± 1.1) than for the HI group (8.0% ± 1.2; p = 0.005). At 6 months, the mean BG levels (mmol/l ± SD) were significantly lower (p < 0.016 for all comparisons) in the IAsp vs. HI group after breakfast (8.8 ± 4.0 vs. 10.6 ± 4.4) , after lunch (7.7 ± 3.4 vs. 9.2 ± 4.0), and after dinner (8.5 ± 3.5 vs 9.5 ± 3.7), as well as before lunch (7.0 ± 3.3 vs. 7.8 ± 4.2). Before dinner blood glucose levels were higher in the IAsp group (8.6 ± 3.7 vs. 8.0 ± 3.8 mmol/l; p < 0.02). The prandial blood glucose (BG) increment (mean difference in pre- and 90 minute post-prandial blood glucose) was significantly smaller in the IAsp group (0.2 ± 2.5 mmol/l) than in the HI group (1.6 ± 2.6 mmol/l; p < 0.0001). The safety of IAsp was comparable to HI, and low frequencies of hypoglycemic episodes were reported for both treatments. The relative risk of experiencing major nocturnal hypoglycemia was 50% (95% CI: 29 - 86%) with IAsp compared to soluble HI. **Conclusion:** IAsp use resulted in lower mean HbA_{1c}, lower mean post-prandial blood glucose levels, and reduced the risk of major nocturnal hypoglycemia when compared to HI.

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EFFECT OF THE INSULIN ANALOGUE INSULIN ASPART ON QUALITY-OF-LIFE AND TREATMENT SATISFACTION IN TYPE 1 DIABETIC PATIENTS.

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Aims: In a 6-month multicentre, multinational, randomised, open-labelled trial on the efficacy and safety of the rapid acting insulin analogue Insulin Aspart (IAsp) in type 1 diabetic patients, quality-of-life and treatment satisfaction were evaluated as crucial outcome parameters. **Methods:** 424 patients (46% females, age 38±12 years, diabetes duration 15±11 years, HbA1c 7.5±1%; mean±SD) from German speaking countries were randomised (ratio 2:1) either to treatment with IAsp (n=283) or regular human insulin (HI; n=141). All patients were on a multiple-injection treatment regimen with IAsp or HI as meal-related and NPH as basal insulin. Only patients on HI were advised to keep an injection-meal-interval of 30 minutes. Two validated treatment satisfaction and diabetes-related quality-of-life scales assessed patients' individual treatment goals, physical complaints, worries about future, social relations, leisure time flexibility, daily hassles, nutritional restrictions, fear of hypoglycaemia, blood glucose fluctuations, self-efficacy, fear of insulin analogues, and burdens of hypoglycaemia. **Results:** After 6 months, IAsp revealed significantly better scores in 2 different treatment satisfaction scales (p<0.005) and quality-of-life with respect to nutritional restrictions (p<0.001). Improved satisfaction was mainly due to higher nutritional and leisure time flexibility (p<0.001). 23% of the IAsp group, and 14% of the HI group achieved small but important improvements of total quality-of-life (p<0.06; criteria according to Evidence-based Medicine Working Group). The *number needed to treat* (NNT) for an important increase in quality-of-life was 10. Regression analysis of potential predictors of quality-of-life improvement indicated that patients who were intensely striving for physical strength (p<0.01; NNT=7) or who felt less protected against hypoglycaemia (p<0.001; NNT=8) were most likely to achieve an important benefit from IAsp.
Conclusions: Under these study conditions, IAsp improved treatment satisfaction and quality-of-life regarding nutritional restrictions. The different NNT indicate that the effect of IAsp on quality-of-life is not trivial.

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HUMAN INSULIN ANALOG, INSULIN ASPART, IS A MEALTIME INSULIN COMPARABLE TO HUMAN INSULIN IN TYPE 2 DIABETES

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Aims: The safety and efficacy of the rapid-acting insulin analogue, insulin aspart (IAsp), with a more rapid onset and shorter duration of action, was compared to human insulin (HI) in a 6-month, multi-center, randomized, open-label, parallel clinical trial in subjects with insulin-requiring Type 2 diabetes. Because IAsp can be given immediately before meals, it provides increased flexibility and potentially improved compliance of treatment for individuals with Type 2 diabetes. **Materials and Methods:** All 182 patients had diabetes for at least 2 years, and had been treated with HI for at least 12 months. At baseline there were no differences between the groups in terms of age, diabetic duration, or glycaemic control (HbA_{1C} = 8.1% ± 1.2% for IAsp and 7.9% ± 1.1% for HI (mean ± SD). Following randomization all patients received bedtime NPH insulin, and bolus injections of either IAsp injected immediately before the meal (n = 91) or HI injected 30 minutes before the meal (n = 91). Efficacy and safety was evaluated at 6 months. **Results:** After 6 months of treatment, HbA_{1C} levels were similar in both groups (7.8% ± 1.3% for IAsp and 7.7% ± 1.1% for HI, p = 0.369). IAsp was also comparable to HI as measured by 8-point BG profiles, prandial BG increments, BG variability measures, and daily insulin dose. The risk of minor or major hypoglycemic episodes was not significantly different for the two treatments. In terms of adverse events, IAsp was as well tolerated as HI, with a slightly higher frequency of non-serious adverse events. The majority of adverse events were mild, and considered unrelated to treatment. **Conclusions:** IAsp injected immediately before the meal is comparable to HI injected 30 minutes before the meal with regard to glycaemic control and the risk of hypoglycaemia in insulin-treated individuals with Type 2 diabetes.

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POSTPRANDIAL ADMINISTRATION OF THE RAPID-ACTING INSULIN ANALOGUE INSULIN ASPART IN TYPE 1 DIABETIC PATIENTS

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Aims: We evaluated prandial glycaemia in type 1 diabetic patients after subcutaneous injection of human regular insulin (HI) and the rapid-acting insulin analogue, insulin aspart (IAsp) at different time-points. **Materials and Methods:** In a randomised, double-blind, double-dummy, 4-period crossover study 20 type 1 diabetic patients (12 male; age 36.4±11.2 (mean±SD) years; BMI 25.0±2.9 kg/m^2; HbA_{1c} 7.6±0.8 %) were investigated. Prandial insulin was administered 15 min before the start of the meal [$HI_{(-15min)}$], immediately before the meal [$HI_{(0min)}$; $IAsp_{(0min)}$] and 15 min after the start of the meal [$IAsp_{(+15min)}$]. To optimise basal insulin requirements patients were admitted the evening before the test meal and received an overnight i.v. insulin infusion to keep blood glucose levels constant at 120 mg/dl. This insulin infusion (optimised basal rate) was maintained till the end of the experiment. The standardised test meal (breakfast at 8.30 a.m.) consisted of 543 kcal (55% carbohydrates, 17% protein, 28% fat). Dosages of s.c. HI and IAsp were kept constant for a single patient during the 4 experiments (mean 9 IU; range 6-12 IU). **Results:** Blood glucose excursions from baseline levels during the 4 hours [$BGexc_{(0-4hrs)}$] were highest with $HI_{(0min)}$ (353 mg·dl^{-1}·h^{-1}; *p<0.05* vs. other treatments). However, $BGexc_{(0-4hrs)}$ were not different for $HI_{(-15min)}$, $IAsp_{(0min)}$ and $IAsp_{(+15min)}$ (266 vs. 248 vs. 282 mg·dl^{-1}·h^{-1}; *p=n.s.*). Maximum concentration of blood glucose (BG_{max}) was lowest with $IAsp_{(0min)}$ (209 mg/dl; *p<0.05* vs. other treatments). BG_{max} was comparable for $HI_{(-15min)}$, $HI_{(0min)}$ and $IAsp_{(+15min)}$ (243 vs. 262 vs. 243 mg/dl; *p=n.s.*). No differences among the treatment groups were observed in terms of basal blood glucose, plasma insulin levels at baseline and areas under the curves of the insulin profiles during the 4 hours after the meal. **Conclusions:** With regard to prandial glycaemia $IAsp_{(+15min)}$ is as effective as $HI_{(-15min)}$ and superior to $HI_{(0min)}$. Thus, postprandial dosing of insulin aspart offers an attractive and feasible therapeutic option for well-controlled type 1 diabetic patients.

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WHAT ARE THE INSULIN REGIMENS USED IN FRANCE ?

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Aims: The current practice for care of patients with diabetes in France is not precisely known. This survey aims at describing how French diabetes specialists manage insulin-treated patients and the metabolic results they obtain. Methods: "Enquête Schéma" was a transversal national survey. During one day in Nov. 98, all diabetologists were asked to answer to an anonymous questionnaire. Results: Among the 934 physicians who were accepted to participate, 450 responded. Physicians' characteristics (which can be shown to be representative of the whole population) were: age: 43.1±7.5 yrs ; 51.9% female ; hospital: 48.9% ; exclusive private practice: 24% ; number of patients seen on the survey day: 2.9±2. Patients' characteristics were: n=1263, all insulin-treated ; type 1 : 57.6% (age: 37.3±19.9 yrs ; diabetes duration: 13.7±11.5 yrs ; insulin treatment duration: 12.3±11.1 yrs ; complications: 36.4%) ; type 2: 36.8% (age: 64±11.4 yrs ; diabetes duration: 14.6±8.9 yrs ; insulin treatment duration: 3.4±4 yrs ; with orals agents: 39.7% ; complications: 63.1%).

Metabolic results

HbA1c	< 7.5%	7.5 - 8.4%	≥ 8.5%
type 1	32.3%	30.1%	37.7%
type 2	34.7%	24.7%	40.7%

Insulin regimens

Type 1 patients				
Injection #/ day	1	2	3	4
%	2.7	42.8	41.2	13.3
Type of insulin*	PM	R+PM	R+NPH	R+L
%	20.1	12.1	33.2	16.5

Type 2 patients			
1	2	3	4
25.7	56.9	14.2	3.3
PM	NPH	other	
32.1	37.7	30.2	

*PM:premixed, R:rapid-acting (regular or Lispro), L:long-acting insulin.

Conclusion : In this survey, 54.5% of the type 1 patients treated by a French specialist used a multiple daily injections regimen (≥ 3) but only 17.5% of type 2 patients used this regimen. According to the DCCT and/or UKPDS criteria, only 1/3 of patients were well-controlled with a large number of patients experiencing complications.

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PHARMACODYNAMICS (PD) AND PHARMACOKINETICS (PK) OF SUBCUTANEOUS INSULIN AND INSULIN LISPRO IN 3 ETHNIC GROUPS.

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Aims: Ethnic differences exist in the epidemiology and clinical features of diabetes, but no ethnic differences have been described in the PD or PK of insulin treatment. This study defined profiles for subcutaneous (SC) regular human insulin (Reg) and insulin lispro (LP) in Caucasian (Ca), Chinese (Ch) and Indian (I) subjects. **Materials and Methods:** Ten healthy, nondiabetic, subjects were recruited from each group. Groups were comparable for age, sex, body mass index, and training level. A randomised cross-over study was performed using a euglycaemic clamp for 8 h after 0.15 IU/kg SC of Reg or LP. Time of onset (Tonset), maximum infusion rate (Rmax), time to Rmax (TRmax), and total glucose infused (Gtot) were derived from the glucose infusion rate vs time curve. Samples were collected for serum insulin, and PK parameters were calculated using noncompartmental techniques. **Results:** LP had more rapid effect than Reg in all groups. In I the PD profile was significantly delayed, most notably with Reg. Peak effect in I also appeared less intense, but total glucose infused was not different. PK did not explain these results.

Rx	Parameter	Indian (I)	Chinese (Ch)	Caucasian (Ca)	P (overall)
Reg	Rmax (mg/kg/min)	3.9 ± 1.5	5.4 ± 3.9	6.5 ± 2.1	0.10
	TRmax (min)	258.0 ± 46.3	198.0 ± 54.0	153.5 ± 53.4	0.0004
	Tonset (min)	49.0 ± 8.5	34.8 ± 7.4	39.6 ± 8.6	0.002
	Gtot (mg/kg)	551 ± 236	642 ± 390	680 ± 221	0.66
LP	Rmax (mg/kg/min)	4.9 ± 2.9	6.2 ± 3.5	8.3 ± 1.7	0.03
	TRmax (min)	113.5 ± 36.4	81.5 ± 41.4	108.0 ± 47.0	0.14
	Tonset (min)	33.5 ± 9.2	25.7 ± 5.9	22.4 ± 6.0	0.006
	Gtot (mg/kg)	605 ± 327	654 ± 336	753 ± 233	0.55

Conclusions: A delay in onset of action and peak effect occurred in I subjects after SC insulin and may have implications for the timing of preprandial Reg insulin for I patients. The delayed profile appeared less pronounced with LP.

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OPTIMIZATION OF POSTPRANDIAL BLOOD GLUCOSE WITH LISPRO INSULIN IN T2DM.

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To assess the effects of lispro (LP) vs human regular insulin (Hum-R) on postprandial (PP) plasma glucose (PG) in type 2 diabetes (T2DM), 11 T2DM patients (age 50±4, mean ± SEM, BMI 26.1±0.3 kg/h^2 on oral hypoglycemic agents, HbA_{1c} 7.6±0.8%) were studied on 4 random occasions (#1 no insulin, #2, #3 and #4, 0.15 U/kg s.c. either Hum-R at 5 min, or 30 min, or LP at 5 min) for 7 hours (h) after a mixed meal (793 Kcal, 60% CHO, 15% proteins, 25% lipids) at 12:30 h (0 h). Six nondiabetics were also studied with the test meal. Areas under curve (AUC) of PG (mg/dl/min) were (*p<0.05 vs #2):

	0-7h	0-4h	4-7h
Nondiabetics	102±4	109±3	90±2
T2DM #1	200±7	208±8	183±9
T2DM #2	169±7	179±8	150±8
T2DM #3	150±5	162±7	127±6
T2DM #4	147±5*	154±6*	139±5*

Hum-R improved PP-PG if given 30 min before the meal vs mealtime administration. LP decreased PP-PG vs Hum-R at mealtime, but not vs Hum-R 30 min because PG-AUC 4-7 h was greater with LP vs Hum-R 30 min (p<0.05). Plasma insulin peaked earlier, but waned earlier; plasma proinsulin, glucagon and C-peptide (C-pep) were all more suppressed in #4 vs #2 and #3 (p<0.05). The late PP-PG (PG-AUC 4-7 h) with LP was lower in T2DM patients with greater C-pep (r=-0.76, p<0.05). Conclusions: in T2DM, PP-PG improves if s.c. Hum-R is anticipated vs meal ingestion. LP always improves PP-PG in early phase (0-4h), but in late phase (4-7 h) only in T2DM with C-pep >0.7 nmol/l. "Basal" insulin is required in T2DM patients with C-pep <0.7 nmol/l to improve interprandial PG and long-term, HbA_{1c}.

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TREATMENT WITH INSULIN LYSPRO WITH CSII CHANGES THE INSULIN AND BLOOD GLUCOSE PROFILE BUT DOES NOT AFFECT PLASMA CONCENTRATIONS OF IGF-1 AND IGFBP-1

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Aim: Changes in the GH,IGF-1, axis with low circulating IGF-1 have been described in patients with Type 1 diabetes. The aim was to study if changes in the insulin profile obtained by treatment with either insulin lyspro or human regular insulin with CSII have different effects on plasma concentrations of IGF-1 and IGFBP-1.

Patients and methods: Twelve patients with Type 1 diabetes, age 47.8 ± 2.4 (mean±SEM) years, body weight 75.7 ± 3.0 kg, diabetes duration 30.5 ± 3.2 years who had been treated with CSII for 12 ± 0.8 years participated in this open label, randomized cross-over study. The patients received their usual bolus insulin dose and a standardized breakfast in the morning. Blood profiles of free insulin, glucose and IGFBP-1 were measured from 7.10 to 14.30 hours.

Results: Insulin lyspro gave a marked peak of free insulin with maximum concentration at 8.00 hrs of 135 ± 20 pmol/l while human regular insulin gave a wide plateau at about 50 pmol/l and no obvious peak. The AUC of free insulin from 7.10 - 9.10 hrs was 11807 ± 1671 during lyspro and 4860 ± 591 (p<0.001) and no significant difference during the later part of the profile. During lyspro the postprandial rise of blood glucose was smaller and the total AUC lower than during regular insulin, 3505 ± 446 and 5087 ± 465 repectively (p=0.006). HbA1c was 6.4 ± 0.2 % on both treatments (ns). There was no significant difference in plasma IGF-1 concentrations, 78.8 ± 10.6 vs 82.3 ± 10.5 µg/l. The total IGFBP-1 AUC was 3678 ± 485 during lyspro and 3860 ± 495 during regular insulin (ns) and there was no difference when dividing the profiles from 7.10-9.10 and 9.10-14.30 hrs.

Conclusions: Insulin lyspro given by CSII gives a different insulin profile and lower postprandial rise of blood glucose than human regular insulin but similar HbA1c. In spite of these differences there is no change of the plasma concentrations of IGF-1 and IGFBP-1.

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Lys(B28),Pro(B29) insulin analogue improves metabolic control in type I diabetic subjects treated by Continuous Subcutaneous Insulin Infusion (CSII).

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Aims: use of the rapidly absorbed lispro insulin analogue in type 1 diabetes improves early post-prandial blood glucose control. However, due to lispro insulin shorter plasmatic peak, patients often experience between meals hyperglycemia. This should not be the case in CSII treated diabetic patients who should gain the most benefit from lispro insulin treatment. The present study was therefore undertaken to investigate whether use of lispro insulin could improve metabolic control in CSII treated type 1 diabetic patients. **Methods:** 39 type 1 diabetic patients (M/F= 17/22, age 24.6±3.5 yrs, BMI= 24.7±2.1 kg/m2) who had been treated by CSII for at least 5 years where asked to record their 9 points daily blood glucose profile twice a week for nine months, while on regular insulin for the first 3 months, on lispro insulin for the following 3 months and back on regular insulin for the final 3 months. HbA1c levels were measured at the end of each three months period. **Results:** HbA1c levels (7.10±0.4% after the first 3 months) dropped to 6.32±0,37%, p< 0.0001 with lispro treatment. They increased back to 7.14±0.36% after the final 3 months of regular insulin treatment (p<.0001 vs lispro). The mean coefficient of variation of blood glucose values at the same time points in different days was significantly lower during lispro insulin than during both regular insulin treatments (7.1±0.53 vs 8.9±0.71; p<.001) and so was the frequency of hypoglycemia (9.7±1.9 vs 11.1±2.1 episodes per 30 days, p< 0.01). **Conclusions:** we conclude that use of lispro insulin in CSII treated type 1 diabetic patients allows achievement of optimal blood glucose control without increased risk of hypoglycemia.

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DOES INSULIN LISPRO ALLOW IDDM PATIENTS THE LUXURY OF AN OCCASIONAL GASTRONOMIC TREAT BETWEEN MEALS?

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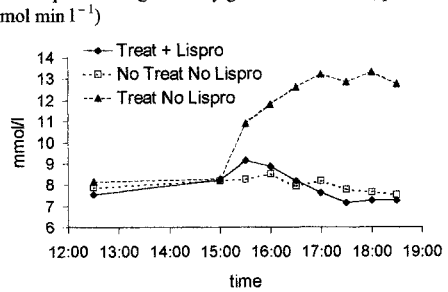
Aims: Contrary to advice diabetic patients may still indulge in the occasional non-diabetic treat. We investigated if an extra dose of Lispro before consumption of a non-diabetic high carbohydrate treat between meals could attenuate glucose excursion on an intensive regimen of Lispro pre-meal and pre-bed Isophane insulin. **Methods**: 8 IDDM patients on intensive therapy aiming for pre and post prandial levels of glucose control between 4 – 10 mmol/l, measured capillary glucose on up to 6 occasions half-hourly for 3 hours after a self chosen treat (e.g. chocolate bar) eaten mid-afternoon with a predetermined extra dose of Lispro. **Results**: Mean AUC for days with treat and extra Lispro were similar to days without a treat or extra Lispro. 1462 ± 42 vs. 1458 ± 40; p= 0.99. AUC on days where the treat was taken without extra Lispro were significantly greater 2169 ± 132; p < 0.001 (mean ± SEM, mmol min l^{-1})



Conclusions: The lack of hypoglycaemia suggests a small extra dose of Lispro can safely control glucose when given with a between meal treat, thus allowing IDDM patients, like non-diabetics, such an occasional luxury.

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EVALUATION OF THE ANTIOXIDATIVE EFFECT OF INSULIN LISPRO THERAPY IN IDDM PATIENTS

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Oxidative stress is an important pathogenetic mechanism in diabetic complications.
Aim: To evaluate the effect of insulin Lispro (LP) on the antioxidative status in IDDM patients, previously treated with human regular insulin (HR).
Materials and methods: Twenty patients with mean age - 28±7.1 years, duration of diabetes - 7.85±4.59 years, BMI - 22.6±2.5kg/m^2 were recruited along with age, sex, and smoking-status-matched control subjects. The glycaemic control was assessed by HbA_1c, blood glucose excursions (BGE) 1-and 2hr after standard meal (400kcal, 15g carbohydrates, 12g fat, 16g protein) at baseline and 3 months after LP treatment. Simultaneously the plasma antioxidant status was measure by thiobarbituric acid reacting substances (TBARS), superoxide dismutase activity (SOD), total antioxidant capacity (TAOC).
Results: The levels of: HbA_1c (4.8±0.5%) and TBARS (2.07±0.65nmol/l) were significantly lower, but the SOD (471.20±53.70U/l) and TAOC (74.10±20.74U/l) were significantly higher in the controls compared to IDDM patients ($p<0.05$). Postprandial 1-and 2hr BGE were significantly lower after 3 months LP therapy compared to baseline ($p<0.001$). For the same period HbA_1c level was not change significantly (8.12±1.64 vs 8.59±1.22%; $p>0.05$). The TBARS significantly decreased after 3 months vs baseline (3.36±0.65 vs 4.09±1.40 nmol/l; $p< 0.05$). The SOD increased significantly at the end of the observation as compared to baseline (444.33±68.8 vs 324.66±51.4 U/l; $p <0.05$). The TAOC increased after 3 months LP therapy vs baseline (44.24±14.1vs 41.79±19.7 U/l).
Conclusions: The insulin LP therapy significantly reduced the postprandial BGE compared to the HR insulin as a result of which the plasma antioxidant status in IDDM patients was improved.

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USE OF HUMALOG® MIXTURES IN THE ELDERLY: A SUBGROUP ANALYSIS OF SAFETY COMPARED TO HUMAN INSULIN MIXTURES.

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Aims: To examine the safety of Humalog® Mixtures in patients greater than 65 years of age compared to human insulin mixtures with respect to incidence of adverse events and hypoglycemia.
Methods: A subgroup analysis was performed on pooled safety data from 4 multinational clinical trials involving Humalog® Mixtures and human insulin mixtures (duration of exposure between 3 and 12 months). Patients were categorized into two age groups (<65 years of age or ≥65 years of age) and comparisons were made between-treatments and between age groups with respect to treatment emergent adverse events (AE = percentage of patients experiencing one or more events) and hypoglycemia rate (episodes per patient per 30 days).
Results: No significant between-treatment or between age group differences were observed in the median hypoglycemia rate or in the overall incidence of treatment-emergent adverse events.

	Humalog® Mixtures			**Human Insulin Mixtures**			p-value
Age	N	Hypoglycemia Rate Median (Mean ±SD)	AE (%)	N	Hypoglycemia Rate Median (Mean±SD)	AE (%)	Hypoglycemia Rate/AE
≥65	66	0.0 (1.4±2.7)	61	63	0.0 (0.9±1.7)	57	NS/NS
<65	330	0.9 (2.1±3.5)	55	323	0.5 (2.0±3.2)	59	NS/NS
p-value		NS	NS		NS	NS	

Conclusions: The safety of Humalog® Mixtures in patients 65 years or older as assessed by the hypoglycemia rate per patient per 30 days and the incidence of adverse events is not different than that of human insulin mixtures. In addition, the safety of patients in the elderly sub-population as compared to the younger population is not different following treatment with Humalog® Mixtures or human insulin mixtures.

904

IMPROVED GLUCOSE CONTROL FOLLOWING ADMINISTRATION OF A 75% INSULIN LISPRO/25% NPL MIXTURE IN PATIENTS WITH TYPE 1 DIABETES.

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Aims: Insulin lispro (L) provides superior blood glucose (BG) control during the first 3-4 hours following meals in comparison to human regular insulin (RI). However, adequate basal insulin replacement is necessary to provide optimal BG control throughout long between-meal intervals. We evaluated HM, a mixture of 75% L and 25% NPL (an intermediate-acting protamine-based formulation of L) with respect to BG control during a 7-hour period following a standard test meal. Comparisons were made to BG control provided by L and RI. **Materials and Methods:** A 3-way, randomized, single dose, crossover study was performed in 31 patients with type 1 diabetes. After an overnight fast, an intravenous infusion of RI was used to standardize blood glucose to 135 mg/dL, which was discontinued 15 min after dosing the study insulin. At 3 separate visits, L, RI, or HM was given subcutaneously before a standard meal (770 kcal; 57% CHO, 29% fat, 14% protein), with L and HM given just prior to the meal and RI given 30 min before the meal. Blood samples were collected every 30 min for measurement of BG over a 7 h period following the start of the meal. The peak BG concentration (BGmax, mg/dL) and mean BG (mnBG, mg/dL) at 4, 5, 6, and 7 h after dosing were assessed. **Results:** BGmax and all mnBG measurements were statistically greater for RI when compared to HM and L. mnBG comparisons were similar for HM and L up to 5 h after dosing, but significantly lower for HM beyond 5h.

	BGmax	mnBG, 0-4 h	mnBG, 0-5 h	mnBG, 0-6 h	mnBG, 0-7 h
RI	•215±60.0	*174±52.8	*176±54.3	*179±55.4	*182±56.3
HM	193±42.1	140±38.9	140±41.3	•142±43.6	•145±45.6
L	185±34.0	141±39.1	148±42.5	156±45.1	165±46.0

*$p<0.05$ when compared to HM, L •$p<0.05$ when compared to L

Conclusions: L and HM provided better BG control than RI during the 7-hour observation period. HM provided better BG control than L when the final 2 hours of the 7-hour observation period were considered.

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POSTPRANDIAL AND PREPRANDIAL ADMINISTRATION OF HUMALOG® MIX25 PROVIDE COMPARABLE GLYCEMIC CONTROL.

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Aims: Humalog® Mix-25 (Mix25), a mixture of 25% insulin lispro (L) and 75% NPL (an intermediate-acting protamine-based formulation of L) recently received approval for marketing in Europe. L has been shown to provide adequate postprandial blood glucose (BG) control when given within 20 minutes after a meal. The administration of Mix25 after a meal was evaluated in this study. **Materials and Methods:** 20 patients with type 1 diabetes were given Mix25 immediately before (Mix25 0)and 20 minutes after (Mix25 +20) a standard meal in a single-dose, randomized, crossover fashion. After an overnight fast, an intravenous infusion of regular human insulin was utilized to standardize blood glucose to 135 mg/dL. The infusion was discontinued 15 min after administration of Mix25. At 2 separate visits, Mix25 was given subcutaneously immediately before a standard meal (315 kcal; 60% CHO, 21% fat, 19% protein) or 20 min after the meal. Serial blood samples were collected for measurement of BG over a 5-h period. BG control was assessed by the peak BG concentration (BGmax, mg/dL), the average BG (BGavg, mg/dL) over a 5 h period after the meal, the time BG was greater than 180 mg/dL (TBG_{180}, h), and the 2 h postprandial BG (BG_{2h}, mg/dL). Comparisons were made by an analysis of variance with a significance level of $p<0.05$. **Results:** The table summarizes mean±SD values for all parameters. No statistically significant differences were detected between Mix25 0 and Mix25 +20.

Treatment	BGmax	BGavg, 0-4 h	BGavg, 0-5 h	TBG_{180}	BG_{2h}
HM-25, 0	233±31.2	182±36.1	172±40.8	2.06±1.34	198±41.5
HM-25, +20	247±36.4	189±41.3	179±47.5	2.44±1.69	197±57.6
p	0.44	0.93	0.95	0.71	0.97

Conclusions: Mix25 given 20 min after a standard meal resulted in postprandial BG control that was not different from that observed following immediate pre-meal administration.

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Insulin Secretion Enhancers I

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NATEGLINIDE ENHANCES THE PHYSIOLOGICAL INSULIN RESPONSE TO A MEAL IN TYPE 2 DIABETICS.

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Aims: Nateglinide (NAT) [A-4166] is a new amino acid-derivative, short-acting, mealtime glucose regulator in development for type 2 diabetes. This study explored the potential beneficial interaction of food and drug administration. **Materials and Methods:** Two cohorts in this double-blind, randomized, 2-center study received NAT 60 mg, then 180 mg (cohort 1, *n*=7), or 120 mg, then 240 mg (cohort 2, *n*=7) in two 7-day periods separated by at least 72 h. 3 more subjects in each cohort received placebo. NAT was given 3 times/day, 10 min before meals. Glucose and insulin were measured on day –1 (fed), after NAT on day 1 (fed), and day 4 (fasting). **Results:** All NAT doses were well tolerated without reports of hypoglycemia. All doses caused rapid, short-lived insulin release and effectively controlled mealtime glucose excursions compared with placebo. The integrated insulin response from predose (AUC-R) for 4 h postdose (or meal) is shown below.

Mean ± SEM	Insulin AUC-$R_{(0-4)}$ (μU*h/ml)				
Treatment	placebo*	60 mg	120 mg	180 mg	240 mg
Meal –NAT	59.1 ± 17.4	45.6 ± 14.8	44.9 ± 4.6	28.4 ± 11.3	29.7 ± 13.2
Fasting +NAT	2.1 ± 3.6	7.1 ± 4.6	7.0 ± 4.7	14.4 ± 2.8	5.3 ± 2.0
Meal +NAT	55.4 ± 16.4	46.8 ± 12.3	78.7 ± 19.5	96.7 ± 20.9 [a]	118 ± 28.2 [b]

*Pooled data from both cohorts. [a] $P<.01$ vs 60 mg, [b] $P<.05$ vs 120 mg.

NAT before a meal stimulated insulin secretion dose dependently within each cohort. NAT doses of 120, 180, and 240 mg before meals significantly enhanced insulin secretion compared with meals without NAT ($P<.05$). The insulin response to NAT in fasted subjects was very limited and less dose dependent than with a meal. **Conclusions:** A limited fasting insulin response and synergy of NAT with a meal contribute to a more physiologic regulation of mealtime glycemia and support a favorable safety profile vs sulfonylureas.

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RELATIVE BENEFITS OF EARLY *VS.* TOTAL INSULIN RELEASE ON GLYCEMIC CONTROL IN 2 RODENT MODELS OF TYPE-2 DIABETES

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The relative benefits of increasing insulin early *v/s* increasing total insulin release, to control glycemic excursions following an oral glucose load (1.35 g/kg) were assessed in 2 rodent models of type-II diabetes mellitus *viz.*, the very insulin resistant Zucker fatty (*fa/fa*) rat and the lesser insulin resistant high-fat fed (HF) Sprague Dawley rat. Neither of these 2 animal models had significantly elevated fasting plasma glucose levels, but fasting plasma insulin levels were 12 X and 2X higher, respectively. Nateglinide, a rapid onset short acting insulin secreting agent and glipizide a slower onset longer acting sulfonylurea were administered 5 min before the oral glucose load. Nateglinide rapidly increased insulin levels but only for a brief period of time such that insulin levels from –5 to 0 min. were increased but total insulin release over the course of the 120-min. experiment was similar to those in the controls. Conversely, glipizide, slower in its onset of action, did not increase early insulin release (-5 to 0 min.) but increased total insulin release relative to the controls. Nateglinide was effective in eliminating glucose excursions in both models with no evidence of sustained hypoglycemia. However, glipizide was only partially effective in curbing glucose excursions in the HF animals and produced significant sustained hypoglycemia starting at 60-min post glucose dose. Interestingly, in the more insulin resistant Zucker rat, glipizide was ineffective in curbing glucose excursions despite an overall increase in total insulin release during the 120-min. sampling period. Hence, the data indicate that independent of the level of insulin resistance, stimulating insulin release early and briefly is more effective in controlling glycemic excursions than is increasing total insulin release.

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CLINICAL EFFICACY AND SAFETY OF NOVONORM® IN THE TREATMENT OF CHINESE TYPE 2 DIABETES IN TAIWAN

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Aim: NovoNorm® (repaglinide) is a newly developed oral hypoglycemic agent belonging to the meglitinide family. It is the first prandial glucose regulator with a unique physiological insulin release profile and short- and fast-acting action. In this 6-month, double-blind randomised study, we compared the efficacy and safety of NovoNorm® at two dose levels (0.5 mg and 2.0 mg) with placebo.

Methods: A total of 92 Chinese type 2 diabetes, with mean age 56.2 ± 8.9 years, BMI 25.2 ± 3.1 kg/m^2 and diabetes duration 6.6 ± 5.0 years, were recruited. They were randomised in a 2:3:3 ratio to 1 of 3 groups: placebo (n= 22), 0.5 mg NovoNorm®(n= 36) or 2.0 mg NovoNorm®(n= 34). The trial consists of a 2-week wash out period and a 24-week fixed dosing period. Majorities (78%) were treated or were previously treated with metformin. Although all treatment groups were comparable in demographics, patients in NovoNorm® group had a longer duration of diabetes than the placebo group.

Results: 12 patients in placebo group, 31 patients in 0.5 mg NovoNorm® group and 31 patients in 2.0 mg NovoNorm® group completed the trial. Comparing with placebo, mean HbA1c decreased significantly by 1.20 ± 0.3% and 1.18 ± 0.3% in the 0.5 mg and 2.0 mg NovoNorm® groups, respectively (p< 0.001). Mean FPG was decreased by 1.04 ± 0.6 mmol/l and 1.75 ± 0.7 mmol/l in 0.5 mg and 2.0 mg NovoNorm® groups, with a statistically significant difference between 2.0 mg NovoNorm® and placebo groups (p = 0.009). No significant differences were shown between NovoNorm® and placebo groups in lipid profiles. Body weight was increased significantly in both NovoNorm® groups. The proportions of patients who reported adverse events were comparable between placebo and 0.5 mg NovoNorm® group and slightly higher in the 2.0 mg NovoNorm® group. Almost all the events were mild or moderate, except for two patients (1 each in placebo and 0.5 mg NovoNorm® groups). Hypoglycemia was reported by 11% of patients in 0.5 mg NovoNorm®, 18% in 2.0 mg NovoNorm® and none in the placebo group. However, all the episodes were considered as mild events.

Conclusion: The present study demonstrates that either dose (0.5 mg or 2.0 mg) of NovoNorm® is a safe and effective in improving glycemic control in Chinese type 2 diabetic patients. However there was a dose-response relationship for effect on FPG, body weight, and number of hypoglycemic events.

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THE THERAPEUTIC EFFECT OF REPAGLINIDE COMBINED WITH TROGLITAZONE IN SUBJECTS WITH TYPE 2 DIABETES.

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Aims: This multicenter, 33-week study was designed to evaluate the efficacy and safety of a combination of repaglinide and troglitazone compared to each drug given as monotherapy. **Materials and Methods:** After screening and a 6-week washout period, 259 subjects were randomized to three treatment groups. The study consisted of three segments; forced titration to fixed dose, titration until maximal dose, and repaglinide added to troglitazone. One hundred and eighty six subjects (for whom data is currently available) received either 2 mg (a.c.) repaglinide (n=59), (400-mg o.d.) troglitazone (n=61) or a combination of both (n=66) for the initial 14 weeks of the study. The percentages of subjects not completing the 14-week treatment period were 5%, 16% and 12% respectively. **Results:** From baseline to week 14, mean HbA_{1c}decreased in combination therapy (8.6% to 7.3%) and repaglinide treated (8.9% to 7.9%) groups, however a slight increase was observed in the troglitazone treated (8.6% to 8.7%) group. ANOVA comparison showed that combination therapy was more effective for reducing HbA_{1c} levels than repaglinide (p=0.02) or troglitazone (p=0.0001) alone. Repaglinide alone was superior in reducing HbA_{1c} levels compared to troglitazone alone (p=0.0001). The frequency of hypoglycemia (% with at least one event) was significantly higher in the combination group (36%) compared to repaglinide (19%) or troglitazone alone (3%). No significant change in liver enzyme values was observed in any of the three treatment groups with the exception of two patients (one in troglitazone treatment and one in the combination therapy group) who had elevated liver enzymes values at week 14. **Conclusion:** This study demonstrates that combination therapy (repaglinide plus troglitazone) leads to a better glycemic control when compared to monotherapy with repaglinide or troglitazone. In addition, repaglinide monotherapy was more effective in lowering HbA_{1c} levels as compared with troglitazone.

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EFFICACY AND SAFETY STUDY OF REPAGLINIDE, A FLEXIBLE PRANDIAL GLUCOSE REGULATOR, IN TYPE 2 DIABETES PATIENTS

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Aims: The efficacy and safety of the novel prandial glucose regulator repaglinide (REP) were compared with placebo in OHA-naïve type 2 diabetic patients, who were considered poorly controlled by diet and allowed a flexible meal regimen. **Materials and Methods:** The study had a multi-centre, randomised, placebo-controlled, double-blind design. Patients (mean age 57.4 ± 9 years, BMI 30.3 ± 5 kg/m^2, diabetes duration 3.0 ± 5 years, HbA_{1c} 7.7 ± 1.7%) were randomised to placebo (n = 138) or REP 0.5 mg (n = 270) at mealtimes. After 4 weeks of treatment, the dose of REP was increased to 1.0 mg at mealtimes if fasting blood glucose levels were above 7.8 mmol/l. **Results:** After a total of 16 weeks, REP treatment had reduced HbA_{1c} from 7.8% to 6.6% (ΔHbA_{1c} -1.14%; $p < 0.001$) compared to a reduction from 7.6% to 7.4% with placebo (ΔHbA_{1c} -0.15%; ns). The decrease in HbA_{1c} was unaffected by the meal pattern (2, 3 or 4 meals daily) chosen by the patients. REP was equally effective in lowering HbA_{1c} when analysed according to BMI (<25, 25–30 and >30 kg/m^2) and age (<65, ≥65 years). There was no significant change in body weight in patients treated with REP (mean 83.4 kg at baseline and 83.7 kg after 16 weeks). Minor hypoglycaemia was reported by 17% in the REP group and 3% in the placebo group, and was independent of meal pattern. **Conclusions:** Mealtime dosing with REP is effective in improving glycaemic control in patients with type 2 diabetes who are uncontrolled by diet alone. With this strategy, patients can achieve flexibility in meal pattern without compromising glycaemic control or increasing the risk of severe hypoglycaemia.

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THE EFFECT OF REPAGLINIDE ON TREATMENT SATISFACTION, WELL-BEING, AND HEALTH STATUS IN TYPE 2 DIABETES PATIENTS

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Aims: This study compared treatment satisfaction (WHO DTSQ), well-being (WHO WBQ) and health status (EQ-5D) during repaglinide (REP) (flexible meal-time dosing) and placebo treatment of oral hypoglycaemic agent-naïve type 2 diabetes patients. **Materials and Methods:** The study had a multi-centre, randomised, parallel-group, double-blind, placebo-controlled design. The two treatment groups were comparable for all baseline characteristics. Change from baseline was compared for both groups by Wilcoxon rank sum test. **Results:** Two hundred and nineteen patients in the REP group and 97 patients in the placebo group completed the study. **WHO DTSQ** (treatment satisfaction): Change in overall treatment satisfaction of REP patients was significantly higher than that of the placebo patients (p=0.025). The subscale "Understanding of Diabetes" rose significantly in the REP group compared with the placebo group (p=0.011). After Bonferroni correction this was not statistically significant. 19.9% of the REP patients versus 15.9% of the placebo group reported improved treatment satisfaction over the 16 weeks (p=0.40). **WHO WBQ** (well-being): On the overall well-being score (scale range 0–67) the REP patients improved by 0.6 whereas the placebo group improved by 0.1 (p=0.87). **EQ-5D** (health status and general health): 37% of the REP group and 29% of the placebo group reported better general health, compared with one year previously (p=0.19). **Conclusions:** The majority of the subscales and overall scores increased over time in both groups, suggesting that flexible meal-time dosing is well accepted by formerly naïve type 2 diabetes patients. Moreover, the results indicate that higher treatment satisfaction and understanding of diabetes can be obtained with REP treatment compared with placebo.

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PHARMACOKINETICS OF REPAGLINIDE IN TYPE 2 DIABETES PATIENTS WITH AND WITHOUT RENAL IMPAIRMENT

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Aims: To compare the pharmacokinetics of repaglinide after single and multiple dosing in type 2 diabetes patients with and without renal impairment. **Materials and Methods:** The study had a single-centre, open-label, parallel-group design. Following assessment of creatine clearance (CL_{CR}), 34 patients (aged 45–75 yr) were stratified by degree of renal impairment into three groups (normal [NORM], mild/moderate [MOD] and severe [SEV]). Patients received a single 2 mg dose on days 1 and 5, and three preprandial 2 mg doses on days 2–4. Blood samples were taken for 24-h (day 1) and 48-h (day 5) serum repaglinide profiles. **Results:** AUC decreased with increasing CL_{CR} both on days 1 (p=0.03) and 5 (p=0.01). When SEV patients were excluded, the correlation between AUC and CL_{CR} on days 1 (p=0.88) and 5 (p=0.75) disappeared. The mean AUC values following single (day 1) and multiple dosing (day 5) were similar (approx. 55 ng/ml·h) for NORM and MOD patients. AUC values in SEV patients were approx. 1.5 (day 1) to 1.75 (day 5) times higher compared with the other groups; this was statistically significant. AUC increased from day 1–5 in the three renal function groups; by 5% in NORM (NS), 21% in MOD (p=0.0002) and 27% in the SEV group (p=0.0001). The mean elimination rate (λ_Z) and $t_{½}$ values were similar for NORM and MOD patients on both days 1 and 5 whereas for SEV patients λ_Z was similar on day 1 but lower than both groups on day 5 (p=0.014/0.017 respectively). From days 1–5, there was little change in λ_Z in the NORM and MOD groups but a significant decrease of 0.27/h (p=0.019) in the SEV group, with a corresponding increase in mean $t_{½}$ from 1.5 h on day 1 to 3.6 h on day 5 in this group. The overall safety profile was similar for all three groups. **Conclusions:** The NORM and MOD renal function groups were virtually indistinguishable from one another for all pharmacokinetic parameters studied, while the SEV group diverged significantly with respect to AUC and λ_Z. Thus, patients with mild or moderate renal impairment can be given normal repaglinide treatment, based on pharmacokinetic considerations.

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Insulin Secretion Enhancers II

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THE NOVEL SULFONYLTHIOUREA 3-15, MAINTAINS GLYCEMIC CONTROL IN ZUCKER RATS DURING LONG-TERM TREATMENT

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3-15, a novel sulfonylthiourea, is an ATP-sensitive potassium channel modulator. It has been shown to improve glucose clearance in obese (fa/fa) Zucker rats (a model of insulin resistance) following 5 days of treatment and its efficacy parallels that of metformin. **Aim:** To establish whether glycemic control could be maintained with 3-15 over the longer term, we studied its effects in obese and lean Zucker rats for 28 days. **Methods:** An initial OGTT was performed prior to treatment. Rats were then treated for 28 days by oral gavage with either metformin (300mg/kg/day), 3-15 (0.1mg/kg/day) or control. At the completion of treatment, the lean rats underwent a second OGTT. At the same time, the right jugular vein and was cannulated for an IVGTT in anaesthetised obese rats. Blood was sampled via the vein at regular intervals over 1h. Plasma samples were analysed for insulin by RIA and for glucose by the glucose oxidase method. **Results:** In the lean rat groups, glucose clearance and weight gain did not significantly differ between any of the treatments. In the obese rats, improvement in the glucose clearance was significantly different between groups treated with 3-15 (AUC: 991.602± 87.547) or metformin (1120.436± 103.029) compared to control (1306.22± 40.87, $p<0.05$ ANOVA). However, the basal insulin concentration was significantly decreased in the 3-15 treated group (33.716±7.491 ng/ml) compared to the metformin (54.576±9.516) and control group (57.447±5.371, $p<0.05$ ANOVA). Maximum and 60 min insulin concentrations were also significantly decreased in the 3-15 or metformin treated rats compared to control. Furthermore, the rate of weight gain in 3-15 treated rats was significantly decreased compared to metformin and control. **Conclusion:** These results indicate that 3-15 and metformin are equally beneficial in improving glucose clearance in obese Zucker rats. However, 3-15 appears to be more potent and acts without causing a compensatory increase in basal insulin levels. 3-15 offers the additional advantage of decreasing weight gain in these obese models compared to metformin.

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ON THE INSULINOTROPIC EFFECT OF SODIUM TUNGSTATE. *IN VITRO* STUDY.

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Aims: To gain further insight into the insulinotropic effect of sodium tungstate (NaWo), we have studied: 1) The insulin responses to glucose and to arginine in pancreases from NaWo pretreated rats. 2) The direct effect of NaWo on insulin release in the presence of diazoxide. **Materials and Methods:** The study was performed in the perfused rat pancreas. Perfusate consisted of Krebs-Henseleit buffer supplemented with dextran (4%), albumin (0.5%) and glucose (5.5 mM). **Results:** Pancreases from NaWo pretreated rats (50 mg/day per os, for seven days) showed a greater insulin response to glucose (incremental area:112±14 ng/15 min) than those from untreated rats (53±7 ng/15 min; $p<0.05$). However, this treatment did not modify arginine-induced insulin release (195±6 vs. 218±6 ng/15 min in control experiments; $p=0.8$). When treated rats were subjected to a 5-day wash-out period (i.e., no NaWo administration), their insulin response to glucose was not significantly different from that of control rats (40±9 vs. 35±6 ng/15 min, respectively; $p=0.6$). Finally, the direct stimulatory effect of exogenous NaWo (5 mmol/l) on insulin output (34±2 ng/10 min) was blocked by 300 µmol/l diazoxide (2.8±1 ng/10 min; $p<0.01$). **Conclusions:** 1) Pancreases subjected to chronical treatment with NaWo show enhanced insulin response to glucose but not to an aminogenic stimulus. 2) Prolonged treatment with NaWo does not seem to be toxic to B-cells since the glucose-induced insulin secretion is not altered when the administration of this salt is discontinued. 3) The blocking of the insulinotropic effect of NaWo by diazoxide suggests that this salt might act on the B-cell via the ATP-dependent K^+ channels.

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JTT-608, A NEW HYPOGLYCEMIC AGENT, ACTIVATES β-CELLS IN A STRICTLY GLUCOSE-DEPENDENT MANNER.

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Aims: JTT-608 (JTT) is a new non-sulphonylurea oral hypoglycemic agent, which stimulates insulin release in the presence of an elevated, but not a basal, blood glucose. It is therefore suggested that this drug does not induce hypoglycemia and is a safer hypoglycemic agent than sulphonylureas. However, the mechanisms for the glucose-dependent insulinotropic action of JTT are little understood. In the present study, we examined the effect of JTT on cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in rat pancreatic β-cells. Second, since the islets *in vivo* are perfused with pituitary adenylate cyclase-activating polypeptide (PACAP), a pancreatic peptide, glucagon-like peptide-1 (GLP-1), an intestinal hormone and ACh, parathympathetic transmitter, possible interactive effects of JTT and these neurohormones were investigated. **Materials and Methods:** Single pancreatic β-cells were isolated from Wistar rats and $[Ca^{2+}]_i$ was measured by dualwavelength fura-2 microfluorometry under superfusion conditions. **Results:** In the presence of 8.3 mM glucose JTT (30-1000 µM) increased $[Ca^{2+}]_i$ in single β-cells in a dose-dependent manner, whereas it had little effect at 2.8 mM glucose, thus exhibiting a strict glucose-dependency. JTT often evoked $[Ca^{2+}]_i$ oscillations. These JTT-induced $[Ca^{2+}]_i$ increases were completely inhibited under Ca^{2+}-free conditions and by nitrendipine (NTD), a L-type Ca^{2+} channel blocker. JTT also recruited glucose-unresponsive β-cells into $[Ca^{2+}]_i$ oscillations. When JTT was combined with PACAP, GLP-1 or ACh, the effect of JTT on $[Ca^{2+}]_i$ was markedly enhanced. **Conclusions:** JTT increases $[Ca^{2+}]_i$ in β-cells by stimulating Ca^{2+} influx through L-type Ca^{2+} channels only in the presence of high glucose (5.6-16.7 mM). This mechanism may account, at least partly, for the insulin release by JTT. The strict glucose-dependent ability of JTT to activate β-cells appears to be an advantage over sulphonylureas. The potency of JTT *in vivo* may be increased by the interaction with physiological neurohormones.

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EFFECTS AND SERUM LEVELS OF GLIBENCLAMIDE AND ITS ACTIVE METABOLITES IN PATIENTS WITH TYPE 2 DIABETES

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Aims: To study the effect and serum drug/active metabolite levels of glibenclamide (Gb) in patients on chronic Gb medication.

Material and Methods: 50 patients with type 2 diabetes on regular Gb therapy (1.75-14 mg). Blood samples were taken before Gb intake and 90 min later. A standardised breakfast was served. Insulin-proinsulin levels were determined by ELISA without cross-reactivities. Serum drug and active metabolite (4-trans-OH-Gb (M1), 3-cis-OH-Gb (M2)) levels were determined by HPLC. Fischer's R to Z test was used for correlations, unpaired Student t-test for comparing different dose groups. A p value < 0.05 was considered significant.

Results: Positive correlations between HbA1c and daily dose ($r=0.55$), therapy duration ($r=0.48$) and diabetes duration ($r=0.42$) were found. Negative correlations between dose and insulin +90 min ($r=-0.59$), D-insulin ($r=-0.59$) and D-proinsulin ($r=-0.52$) and between therapy duration and insulin ($r=-0.40$) and proinsulin ($r=-0.34$) secretion were found. There were positive correlations between dose and ratio pro-insulin to insulin molecules (RPI) at both time points ($r=0.32$ and 0.30), and RPI was lower after Gb intake. In patients on >7.0 mg, steady state serum metabolite levels (M1 and M1+M2) were higher than those of Gb itself. No correlation was found between fasting blood glucose and maximum Gb levels.

Conclusions: In patients on chronic medication, Gb is able to stimulate both insulin and proinsulin secretion; the release of insulin was relatively greater. The effect was more pronounced in patients on a low Gb dose. In patients on a daily dose > 7.0 mg, serum metabolite levels of clinical relevance have been demonstrated; the metabolites may contribute to hypoglycaemic events.

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EFFECTS OF AGEING ON GLIQUIDONE, GLIBENCLAMIDE, GLICLAZIDE AND GLIPIZIDE PHARMACOKINETICS

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Aims: To compare the pharmacokinetics of four hypoglycemic sulfonylureas eliminated mainly by either the kidney or liver in middle-aged and aged subjects. **Materials and Methods:** Six middle-aged (48 ± 3 yr; mean ± SEM) and 6 aged (73 ± 1 yr) subjects, all of the latter with normal creatinine clearance, received each, on separate days, an oral administration of gliquidone (30 mg), glibenclamide (5 mg), gliclazide (80 mg) and glipizide (5 mg). The plasma concentration of the drugs was measured before and at 8 times (60 min to 24 h) thereafter by a standardized HPLC procedure for the identification and assay of hypoglycemic sulfonylureas. **Results:** In middle-aged subjects, the peak concentrations for gliquidone, glibenclamide, gliclazide and glipizide averaged, respectively, 0.81 ± 0.07, 0.14 ± 0.01, 4.76 ± 0.63 and 0.41 ± 0.06 mg/l, with corresponding peak times of 1.58 ± 0.08, 2.58 ± 0.37, 4.17 ± 0.65 and 2.08 ± 0.40 h and half-lives of 1.61 ± 0.10, 2.63 ± 0.75, 6.96 ± 2.11 and 1.94 ± 0.45 h. The half-life of gliclazide was thus higher than that of the other 3 hypoglycemic agents in the middle-aged subjects. It was also the sole to be significantly increased ($P < 0.05$) in aged subjects, to 20.83 ± 6.31 h. **Conclusions:** There is no obvious difference between sulfonylureas eliminated mainly by either the kidney (glibenclamide, gliclazide, glipizide) or the liver (gliquidone) in terms of the influence of ageing upon their clearance, at least in subjects with normal creatinine clearance.

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GLUTAMATE STIMULATES INSULIN SECRETION AFTER ORAL GLUCOSE LOAD IN HUMANS.

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Glutamate has been shown to stimulate glucose-induced insulin secretion *in vitro* by acting on an excitatory amino acid receptor of the AMPA subtype. It was also shown to improve glucose tolerance *in vivo* in the rat. We performed a double-blind placebo-controlled cross-over study to investigate the effects of glutamate on plasma insulin and glucose concentrations during an oral (75 g) glucose tolerance test in healthy volunteers. Monosodium L-glutamate (10 g) was administered orally to 18 subjects (13 men and 5 women) with a mean age of 22 years (range: 19-28), after informed consent was given; one of the subjects did not complete the trial. Glutamate was assayed by high performance liquid chromatography, insulin and C-peptide by radioimmunoassay and glucose by the enzymatic glucose oxidase method. The increase in plasma insulin and C-peptide concentrations after glucose absorption was slightly higher, although not significantly, after glutamate administration than after placebo. However, the areas under the insulin and C-peptide concentration/time curves for 75 min (AUC_{75}), corresponding to the t_{max} of glutamate kinetics, were significantly correlated with the AUC_{75} of glutamate concentrations ($r = 0.485$, $P = 0.049$, and $r = 0.611$, $P = 0.009$ respectively, $n = 17$). There was a great variability in plasma glutamate concentrations and subjects could be classified in two subgroups according to glutamate bioavailability. In the subgroup of subjects with total glutamate AUC ≥ 30% as compared with the placebo period ($n = 9$), there was a significantly higher insulin (+ 30.6%) and C-peptide (+ 17.5%) response versus placebo ($P = 0.03$), which was not the case when glutamate bioavailability was < 30% ($n = 8$). However, glutamate was without significant effect on glucose tolerance. It is concluded that L-glutamate enhances glucose-induced insulin secretion in healthy volunteers in a concentration-dependent manner; since it does not modify the glucose tolerance, the clinical relevance of this pharmacological effect remains to be further assessed.

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BTS 67 582 HAS COMMON AND DISTINCT BETA CELL ACTIONS TO SULPHONYLUREA AND IMIDAZOLINE DRUGS

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Acute and chronic effects of BTS 67 582 were examined in the insulin-secreting BRIN-BD11 cell line. In acute 20 min incubations (n=6), 100-400 µmol/l BTS 67 582 stimulated a 1.6-2.6-fold increase ($p<0.001$) in insulin release at 1.1 mmol/l glucose (1.64 ± 0.06 ng/10^6 cells/20 min, mean ± SEM). Long-term exposure (3-18 h) to 100 µmol/l BTS 67 582 in culture dose-dependently decreased subsequent responsiveness to acute challenge with 200 µmol/l of BTS 67 582 or tolbutamide by 28-44% and 33-50% at 12-18 h ($p<0.001$). In contrast, 18 h BTS 67 582 culture did not significantly affect the insulin-releasing actions of 16.7 mmol/l glucose, 200 µmol/l efaroxan, 10 mmol/l arginine or 30 mmol/l KCl which evoked respective 1.4-fold, 2.6-fold, 3.6-fold and 9.8-fold acute responses ($p<0.001$) after normal culture conditions. However, 18 h culture with BTS 67 582 significantly ($p<0.001$) inhibited the 1.6- and 6.6-fold insulin-releasing actions of 10 mmol/l 2-ketoisocaproic acid and 10 mmol/l alanine (by 24 and 36%, respectively). Consistent with the view that these effects were primarily exerted through early stages of the BTS 67 582 and tolbutamide signalling pathway, BTS 67 582 culture exerted no influence on the respective 3.8 and 9.8-fold responses ($p<0.001$) to 25 µmol/l forskolin and 10 nmol/l phorbol-12-myristate 13-acetate. In conclusion, these data suggest that the guanidine derivative BTS 67 582 shares a common signalling pathway to sulphonylurea but not imidazoline drugs. Furthermore, downregulation of the drug actions may prove an important tool in dissecting the molecular sites of action of novel and established insulinotropic antidiabetic agents.

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Diabetes and Pregnancy I

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INSULIN DOSE DURING STEROID TREATMENT FOR FOETAL LUNG MATURATION IN DIABETIC PREGNANCY - TEST OF AN ALGORITHM

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Aim: Test of safety and efficacy of an algorithm for increment of insulin dose during glucocorticoid treatment for foetal lung maturation in third trimester in insulin dependent diabetic women. **Background:** It is well documented in non-diabetic women that maternal glucocorticoid treatment can increase foetal lung maturation and thus reduce the incidence and severity of respiratory distress syndrome in infants delivered before 34 weeks. Foetal lung maturation is impaired in the offspring of diabetic mothers and preterm labour is significantly increased. Glucocorticoid treatment impairs glucose metabolism and is associated with an increased risk of severe dysregulation with ketoacidosis. Thus this treatment has not been used in diabetic pregnancies in many departments all though one could argue that the offspring of diabetic women from a theoretically point of view could benefit from this treatment. **Material and methods**: All insulin dependent women treated with glucocorticoid in third trimester for foetal lung maturation were identified retrospectively in the period 1996-99. The material consists of one cohort before and one cohort after an algorithm for increment of insulin dose was introduced in 1997. At day 1 and 2 the women received dexamethason 12 mg. im. For the second cohort the insulin dose was increased after the algorithm with 40; 40; 20; 10 % the 2; 3; 4; 5 day after glucocorticoid treatment prior to any increase in blood glucose. Additional insulin injections were given with preprandial levels above 8 mmol/l in both groups. None received tocolytica. **Results:** The two cohorts were comparable n=8, median age 31 vs.31 y., duration of diabetes 16 vs. 14 y. week of gestation 29 vs 28, Haemoglobin A1c 7,0 vs. 6,6%, insulin dose 64 vs. 62 IU/24h. On the 2. to 5. day median blood glucose was increased to 14,3; 12,3; 7,7; 7,7 vs 8,2; 9,6; 7,0; 7,4 mmol/l, respectively ($p<0,05$ between the groups). Insulin dose was increased 38; 36; 27; 17 vs. 45; 40; 31; 11 %, respectively. The number of blood glucose < 3 mmol/l was 0 (range 0-2) vs. 0,5 (0-5). None developed significant ketonuria or severe hypoglycaemia. **Conclusion:** Our algorithm for increment of insulin dose during glucocorticoid treatment for foetal lung maturation in insulin dependent diabetic women seems safe and improve blood glucose control.

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SUCCESSFUL OUTCOME OF PREGNANCY IN DIABETIC PATIENTS TREATED BY IMPLANTED INSULIN PUMP.

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Treatment by implanted insulin pump is still under clinical investigation as the insulin (Genapol-stabilized U400 HOE 21 PH) has not yet completed the marketing approval.So pregnancy cannot be allowed. However several French centers presently report 8 successful albeit unexpected pregnancies in 5 women treated by implanted insulin pump. At the time of pregnancy occurence, patients had a mean age of 30.5 years, a mean diabetes duration of 21 years and late complications as severe retinopathy treated by laser (n=4) and nephropathy (n=2). IP treatment lasted from 6 months to 8 years and had been indicated in all cases because of poorly controlled diabetes under intensive SC treatment. Throughout pregnancy mean HbA1c (normal value measured by HPLC: 5±0.5% (m±SD)) decreased from 6.9 to 6 %. No severe hypoglycemia was reported.Two events of moderate ketosis occured.Mean weight gain was 12.6 kg. Local tolerance of the implanted pump was excellent in 7 cases. In 1 pregnancy, an aseptic seroma occured in the pump-pocket and led to inflammatory cutaneous reaction. Two patients had reimplantation during pregnancy (1 for battery depleted, 1 for slowdown). Planned delivery was induced at 38 weeks of gestation in 5 pregnancies. Mean birth-weight of children was 3560 g. Early caeserian section was performed at 32 weeks in 1 case, 33 weeks in both pregnancies of the patient with hypertension. Birth-weight of children was : 3260, 1600 and 1720 g. Some transient fetal complications were observed: infection and renal insufficiency (n=1), polyglobulia and icteria (n=1), hypospadias (n=1). After pregnancy the retinal and renal status of all mothers remained at pregestational grades. One patient was temporarily explanted for cutaneous problem. Evolution was good for all children. So in 7 out of 8 pregnancies there was no specific problem related to the implanted pump. In these women, pregnancy was unrecommended since unallowed by regulations of clinical trials and submitted to the risk of adverse events due to to basically unstable and complicated diabetes. However implanted insulin pump allowed to achieve good metabolic control and so permitted the successful outcome of pregnancy in these patients with high risk pregnancy.

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MATERNAL AND FETAL OUTCOMES BETWEEN HUMAN REGULAR AND HUMALOG® INSULIN TREATED PREGNANCIES IN TYPE 1 DIABETES

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Aims: Safety and efficacy of Humalog insulin during pregnancy is currently unknown. **Materials and Methods:** Sixty women (32 on Human Regular insulin since 1990 and 28 on Humalog since 1996) were closely followed for their diabetes care and albumin excretion rate-(AER), progression of diabetic retinopathy and development of toxemia of pregnancy and fetal outcomes. **Results:** Twelve out of 32 and eight out of 28 subjects were on multiple insulin shot regimens in Regular and Humalog treated groups respectively and the remainder used insulin infusion pumps. Mean (±SD) age and duration of diabetes in the Regular insulin treated groups was 24.12±4.1 years and 16.5±6.9 years respectively vs. 26.4±4.1 years and 15.9±6.1 years for the Humalog treated group. Mean HbA1c values were significantly lower ($p<0.01$,t-test) throughout the pregnancy in the Humalog treated subjects when compared with Regular insulin treated group (7.3% vs 8.6% around 4 weeks of conception, 6.4% vs 7.3% ~20 weeks of gestation and 6.2% vs 6.8% at the end of the pregnancy respectively). The mean AERs and eye grades were not different in the two groups before, during, or after delivery. However, two subjects needed laser treatment during or immediately after gestation in the Regular insulin treated group. Hypoglycemic episodes were significantly lower during the Humalog treated pregnancies when compared with Regular insulin group (<0.01,t-test). Mean (±SD) of total weeks of gestation was 35±1.7 vs 37.0±2.0 weeks in Regular vs. Humalog treated groups respectively ($p<0.03$,t-test). Apgar score, birth weight, and incidence of toxemia of pregnancy were not different in the two groups. Fetal abnormalities included birth heart defects, craniosynostosis, SIDS, (one each) in Regular insulin group, and partial deafness and cleft lip and palate (one each) in the Humalog treated group. **Conclusion:** Better glycemic control with significantly less hypoglycemia is achieved with Humalog treatment during pregnancy without any adverse effect on diabetic retinopathy, nephropathy, or fetal outcomes.

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PERINATAL "PROGRAMMING" OF INSULIN RESISTANCE: A CRITICAL ROLE FOR NEONATAL INSULIN IN THE „SMALL-BABY-SYNDROME"?

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Aims: Low birth weight is associated with the later development of insulin resistance. The neonatal endocrine situation might play a crucial aetiopathogenetic role, not evaluated in this context so far. Children of mothers with hyperglycaemia during pregnancy are a risk population for later insulin resistance. We examined relationships between birth weight, insulin and insulin resistance at birth, and parameters of insulin secretion and insulin resistance in later life of those children. **Materials and Methods:** Data of 104 infants of mothers with insulin-dependent diabetes mellitus during pregnancy, born between 1980 and 1990 at Berlin-Kaulsdorf, Germany, were analysed. Oral glucose tolerance tests (oGTT; area under the curve of glucose, AUCG) with parallel determination of insulin (area under the curve of insulin, AUCI) were performed at 1-5 years of age. Using correlation and regression analysis, birth data were related to insulin secretion (AUCI) and stimulated insulin/glucose-ratio (AUCI/AUCG) in childhood. **Results:** After adjustment for confounders, birth weight was negatively correlated to AUCI in childhood ($p = 0.03$). Insulin/glucose-ratio at birth was positively correlated to AUCI ($p = 0.02$). Both insulin and insulin/glucose-ratio at birth were positively correlated to AUCI/AUCG at reexamination ($p = 0.04$). The inverse relation between birth weight and AUCI/AUCG in childhood was not significant ($p = 0.12$). In the regression analysis, birth weight was significantly negatively correlated to AUCI/AUCG in childhood only when the insulin/glucose-ratio at birth was considered statistically. **Conclusions:** Neonatal insulin and insulin resistance are positively related to insulin secretion and insulin resistance in later life, in addition to the influence of low birth weight, but independent of it. The neonatal endocrine situation should be considered in future studies on the „small-baby-syndrome".

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MATERNAL LEPTIN, IGF-2 AND IGFBP-3 IN DIABETIC PREGNANCY : RELATIONSHIP TO PREPREGNANCY BMI AND BIRTH-WEIGHT.

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Aims: Maternal leptin, insulin, IGF-2, IGFBP-3 levels and their relationship with pre-pregnancy body mass index (BMI) and birthweight were examined in 56 women, 21 normal pregnants (NP), 22 with impaired glucose tolerance during pregnancy (ITG), 13 with gestational diabetes mellitus (GDM). **Materials and Methods:** Blood samples for leptin, insulin, IGF-2 and IGFBP-3 assay were collected in 4 patients at before 24, in n°42 at 24-32 and in n°7 at 32-40 weeks of gestation. NP, IGT and GDM women showed no difference in the concentrations of leptin (NP=25.75±14.32 ng/ml, ITG=22.05±12.56 ng/ml, GDM=19.17±8 ng/ml), insulin (NP=9.93±6.6 µU/ml, ITG=10.07±5.57 µU/ml, GDM=9.36±4.36 µU/ml), IGF-2 (NP= 593.74±112,82 ng/ml, ITG=641.55±220.87 ng/ml, GDM= 538.71±207ng/ml) and IGFBP-3 (NP=7.67±2.91 µg/ml, ITG=7.66±2.13 µg/ml, GDM= 9.57±4.13 µg/ml). All subjects were divided into two groups based on their pre-pregnancy BMI: less than 25 Kg/m^2(group A, n° 39); 25-35 Kg/m^2(group B, n°16). **Results:** Group B showed leptin values significantltly higher than that of group A (26.35±11.62 ng/ml vs 21.54±12.62, p=0.008), but no difference in IGF-2, IGFBP-3 concentrations. Moreover group B insulin levels were on average 20% higher than that of group A (11.67±6.29 µU/ml, 9.06±5.24 µU/ml respectively). The birth-weight was similar in two groups (A= 3325±405 g; group B= 3379.8±576.41 g). The correlation between serum leptin and maternal pre-pregnancy BMI was significant in all cases (r=0.28, p<0.03). Leptin levels correlated also with insulin levels in both groups (A: r=0.39, p=0.001; B: r=0.72, p=0.001; A plus B: r=0.27, p<0.001), but not with IGF-2 and IGFBP-3. Birth-weight was not correlated with maternal leptin, IGF-2 and IGFBP-3 serum. **Conclusions:** These results indicate that maternal leptin IGF2, IGFBP-3 levels are not influenced by IGT or GDM and that prepregnancy overweight is associated with increased leptin concentrations in pregnancy. Moreover maternal leptin IGF-2 and IGFBP-3 are not indicators of fetal growth as fetal leptin and growth factors levels.

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PREGNANCY OUTCOME IN WOMEN WITH DIABETES MELLITUS TYPE 2 (DM Type 2) IS NOT GOOD.

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Aims: The aim of this study was to examine outcomes i.e. early pregnancy loss <24 weeks, congenital malformations (CMR), stillbirths (SBR), early neonatal deaths (ENND) and perinatal mortality (PMR) in pregnancies complicated by DM Type 2 and compare them to outcomes in pregnancies with DM Type 1. **Methods:** In 1997 we established a computerised database of information on pregnancies complicated by diabetes mellitus. Information was retrospectively collected to 1990 and continues prospectively, with 510 pregnancies recorded to date. **Results:** 57 women with pregnancies complicated by DM type 2 were identified representing 12% of the total database population. 25 were asian, 18 caucasian and 14 Afro-caribbean and other ethnicities. There were 48 live births (83%), 8 spontaneous pregnancy losses before 24 weeks (14%) one termination and one early neonatal death (1.7%). There were no stillbirths. 7 congenital malformations (12%) occured, 6 of which were cardiac. Only 2 women attended for pre-pregnancy care. The mean booking HbA_1C was 9.6% +/- 1.4 (mean +/- SD), and the mean week of antenatal booking was 10.6 +/- 3.56 (mean +/- SD). The early pregnancy loss rate was 140/1000 (Type 2) v 78/1000(Type 1) v 9.8/1000 for the total database population (n=510). The CMR was 122/1000(Type 2) v 78/1000(Type 1).There were no stillbirths v 9.8/1000(Type 1), v 5.8/1000(background population). The ENND rate was less at 17/1000 (Type 2) v 19.6/1000(Type 1) and 9.2/1000(background) as was the PMR at 17/1000(Type 2) v 29/1000(Type 1) v 15/1000 (background). **Conclusion:** Pregnancy outcomes in women with established DM Type 2 are not good. Early pregnancy loss and congenital malformations are almost twice that observed in DM Type 1. Although the SBR, ENND and PMR are better in DM Type 2 than in DM Type 1 they remain excessive when compared to the background population. A fetus of a pregnancy complicated by DM Type 2 is at great risk and this is in part related to poor attendance for pre-pregnancy care, late booking for antenatal care and poor glycaemic control in the critical period of implantation and organogenesis.

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THE USE OF A NOVEL DATABASE SYSTEM FOR AUDITING AND PREDICTING MACROSOMIA IN DIABETIC PREGNANCIES

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Aims: EILEITHYIA is a relational database system for auditing diabetes during pregnancy. Traditional parameters, such as glucose values and glycosylated haemoglobin (HbA_1c), may not be the most appropriate factors for predicting the outcome of a pregnancy complicated by diabetes mellitus. **Materials and Methods:** A cohort of 40 pregnant diabetic women (median age 33.0 (IQR 9.5)) from an inner-city, multi-ethnic population was studied prospectively until delivery. Seven of them had pre-existing diabetes (three (8%) type 1, four (10%) type 2) and 33 diagnosed as having gestational diabetes (82%). **Results:** The incidence of macrosomia, defined as birth weight ≥ 4000 g or centile birth weight ≥ 90% was significantly lower in women who have planned their pregnancies (Mann Whitney U, 3545 g (1200 g) vs 4140 g (645 g), p=0.03 and 53.0% (69.9%) vs 98.5% (8.6%), p=0.04). There was significant correlation between birth weight and planned pregnancy (Spearman's, r=-0.66, p=0.002) and parity (r=0.44, p=0.005), as well as between centile birth weight and planned pregnancy (r=-0.64, p=0.007) and parity (r=0.51, p=0.002). Type of diabetes was significantly correlated with centile birth weight, with heavier babies born to women with pre-existing diabetes (types 1 and 2) than to women with gestational diabetes (Spearman's, r=-0.38, p=0.03). Finally, body mass index (BMI) was significantly correlated with systolic and diastolic blood pressure (Spearman's, r=0.33, p=0.03). **Conclusions:** In women whose pregnancy is complicated by diabetes, unplanned pregnancy and, possibly, the type of diabetes are risk factors for macrosomia. Database systems, such as EILEITHYIA, can facilitate clinical practice and audit and identify predictive factors for outcome.

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PRENATAL OUTCOME AND CLINICO-METABOLIC INDICES IN IDDM PATIENTS BEFORE AND DURING PREGNANCY.

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The aim - to reveal relation between prenatal outcome and clinical and metabolic indices in women with IDDM before and during pregnancy. Materials and metods: A total of 38 pregnat women were included in the study. All the women received preconception care. The study population was devided into 3 groups on the basis of prenatal outcome: Gr. 1 – 16 women (infant birthweigh 3.000 – 3.700g); Gr 2 – 11 women (>3.700-4.000g); Gr 3 – 11 women (<2.400-3.000g). Results: Maternal age: the women in Gr 2 were the oldest (Gr 1 – 20.7±2.4; Gr 2 – 32.3±2.1; Gr 3 – 28.3±2.0; P_{1-3}<0.05, P_{2-3}>0.005). BMI (kg/m2) was the highest in Gr 2 (Gr 2 – 26.6±1.9; Gr 1 – 21.75±0.62; Gr 3 – 20.8±0.58; P_{1-2}<0.005, P_{2-3}<0.01). At conception good glycemic control was achieve, and maintained throughout the pregnancy: HbA1c % - Gr 1 - 7.2±0.3; Gr 2 – 7.1±0.4; Gr 3 – 7.0±0.3. In the 1-st trimester, insulin doses (U/kg), were higher in Gr 2 and 3(0.70±0.06 and 0.70±0.05, respectively), than in Gr 1 (0.52±0.04; P_{1-2}<0.01, P_{1-3}<0.010). In the 3-rd trimester the highest insulin doses were in Gr 2 (0.54±0.05, P<0.001), compared to 0.54±0.05, P<0.001 (Gr 1) and 0.75±0.45; P<0.05 (Gr 3). During the pregnancy the largest weightgain was observed in Gr 3 (1-st trimester – 66.0±4.03 kg, 2-nd trimester – 79.5 ±4.61kg, P<0.05). Microalbumin (MA) was found in 45.4% and 18.7% (Gr 2 and Gr 1, respectivly), and proteinuria in 18.1% of Gr 2 patient. The highest LDLP-CH was registered in Gr 2 by the end of the 3-rd trimester (Gr 1 – 3.11±0.39, Gr 2 – 5.42±0.62; Gr 3 – 3.98±0.52, P_{1-2}>0.01, P_{2-3}<0.05). Conclusion: 1.High BMI indices before conception; age over 30 yrs; weight gain more then 15 kg, insulin dose increase >0.90 U/kg and high LDLP-CH concetracions by the end of pregnancy may be considered as risk factors for excessive birth weight. 2. MA and/or proteinuria may be considered as risk factors for low birth weight. 3. We did not find any relation between the HbA1c levels and infant's birth weight.

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INCREASED GENE EXPRESSION OF CATALASE AND Mn-SOD IN EMBRYOS OF DIABETIC RATS

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Maternal diabetes during pregnancy increases the rate of dysmorphogenesis in the offspring. Excess free oxygen radicals are thought to influence the teratogenic process. In a rat model for diabetic pregnancy, the offspring of a malformation-resistant rat strain ("H") show very little dysmorphogenesis when the mother is diabetic, whereas the offspring of diabetic rats of a sister strain ("U") display marked growth retardation and morphological malformations. The embryonic catalase activity is higher in the H than in the U strain and maternal diabetes increases the difference. **Aims:** To determine if the gene expression of the radical scavenging enzymes catalase, glutathione peroxidase (GSHpx), γ-glutamylcystein-synthetase (γ-GCS), glutathione reductase (GR) and superoxide dismutase (CuZn-SOD and Mn-SOD) was altered by maternal diabetes. **Materials and Methods:** Expression was measured in day 11 embryos of normal and diabetic rats of the H and U strains using semi-quantitative RT-PCR and normalised with respect to β-actin expression. The sex of the embryos was determined by PCR for expression of the SRY-gene. **Results:** The expression of GSHpx, γ-GCS, GR and CuZn-SOD did not display any differences. Maternal diabetes increased ($p < 0.05$, ANOVA) the expression of catalase (0.885 vs. 0.413 with non diabetic mother) and Mn-SOD (3.372 vs. 0.858) in H embryos, but not in U embryos. SRY examination showed that the diabetes-induced difference for Mn-SOD mainly appeared in female H embryos. **Conclusions:** Maternal diabetes increases embryonic expression of radical scavenging enzymes in a malformation-resistant rat strain, in a manner that, for Mn-SOD, appears to be gender specific.

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EFFECTS OF PENTOSE PHOSPHATE PATHWAY INHIBITION IN MOUSE PERI-IMPLANTATION EMBRYOS DEVELOPING IN HIGH GLUCOSE.

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Aims: Increased levels of glucose are known to induce an oxidative stress in rat embryos during organogenesis. Here, we investigate the antioxidant defenses of early mouse embryos (blastocysts) by analysing the role of the pentose phosphate pathway, a metabolic pathway producing the NADPH required for the recycling of oxidized glutathion. **Materials and Methods**: Mouse blastocysts were incubated for 24h in the presence of increasing concentration of D-glucose (1mM, 6mM, 17mM and 28mM) and then analysed for their major metabolic activities: glycolysis, Krebs cycle and pentose phosphate pathway using different glucose radioisotopes. Blastocysts were also incubated in the presence of dehydroepiandrosterone (DHEA) an inhibitor of glucose-6 phosphate dehydrogenase, the first enzyme of the pentose phosphate pathway. **Results:** the production of $3H2O$ from [5-3H]-glucose, which reflects glycolysis activity, decreased significantly with increasing concentration of glucose ($p<0,01$). The activity of the Krebs cycle, measured by the production of $14CO2$ from [6-14C]-glucose, remained unchanged. Analysis of the proportion of glucose metabolized through the pentose phosphate pathway increased at the higher concentration of glucose. In spite of these metabolic changes, the average number of cells per embryo was not affected in high concentrations of glucose. In the presence of DHEA, embryos developing in 28mM glucose showed no change in their cell number. In control embryos (6mM), the same DHEA concentration (100μM) had a toxic effect on cell number. **Conclusions**: Mouse blastocysts were found to up-regulate the activity of the pentose phosphate pathway in response to high glucose in vitro. Inhibition of this pathway with DHEA, however, did not further sensitize the embryos to the influence of high glucose.

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ANALYSIS OF TWO INTRA-CELLULAR EFFECTORS OF APOPTOSIS IN RAT EARLY EMBRYOS EXPOSED TO HIGH GLUCOSE

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Aims: Previous investigations have shown that maternal diabetes already impairs embryo development during the earliest phase of gestation. Embryos exposed to high concentrations of glucose in vitro are characterized by a decrease in the number of cells and a concomitant increase in two markers of apoptosis: chromatin degradation (karyolysis) and nuclear fragmentation (karyorhexis). Here, we show that two intra-cellular effectors, caspase-3 and caspase-activated deoxyribonuclease (CAD), are involved in this apoptotic process. Preliminary observations have shown that these two effectors are expressed in blastocysts. **Materials and Methods:** Rat blastocysts were incubated for 24 hours in either 6mM or 28mM glucose in the presence or absence of an inhibitor. DEVD-CHO (10μM) inhibits the activity of caspase-3 and Aurin (1μM) inhibits CAD. After incubation, blastocysts were examined for the proportion of nuclei showing signs of karyolysis (terminal transferase-mediated dUTP nick end labeling technique) and karyorhexis (bisbenzimide staining). **Results:** Addition of DEVD-CHO was found to inhibit the increase in karyolysis induced by high glucose as well as the low incidence detected in control blastocysts. Addition of Aurin prevented the increase in karyolysis triggered by excess glucose but did not influence the basal apoptotic activity in blastocysts. None of these inhibitors prevented the increase in karyorhexis induced by high glucose. **Conclusions:** Our data indicate that karyolysis and karyorhexis are two nuclear damages that are induced separately by high glucose in rat blastocysts. Karyolysis is apparently mediated by the activation of caspase-3 and CAD. The identity of the apoptotic effector(s) involved in karyorhexis will be investigated in further experiments.

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THE MINIMAL MODEL AND THE HOMEOSTATIC MODEL ASSESSMENT IN PREGNANCY.

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Aim: To assess the correlation of the insulin sensitivity index (Si) derived from the minimal model with insulin sensitivity (%S) derived from the Homeostatic Model Assessment (HOMA) in the third trimester of pregnancy. **Materials and Methods:** Twenty-seven pregnant women had an insulin modified (0.02 U/kg) frequently sampled intravenous glucose tolerance test (glucose 0.3g/kg) at 34 weeks gestation as part of a study investigating the role of maternal metabolism in determining birthweight. Ten women were Obese (O) ($BMI>30kg/m^2$) and 17 were Lean (L) ($BMI<30kg/m^2$). Specific insulin was assayed using an ELISA with a sensitivity of 0.55 $pmol.l^{-1}$ and inter-assay specificity less than 8.6% at low and high values. The cross-reactivity of proinsulin with the intact insulin assay is negligible. Glucose was assayed using an enzymatic method. The Ciba program was used to derive Si using the minimal model and the HOMA programme was used to derive %S. **Results:** The Median (IQR) Si was significantly higher in L = 1.5 (0.8 – 3.4) vs O = 0.6 (0.5 – 0.8) $(1/(min.pmol/l)) \times 10^{-4}$, $p<0.01$. HOMA did not detect a significant difference in %S between L = 168.7 (90.7 – 273.3) vs O = 102.2 (94 – 137.3). Spearman's rank correlation coefficient for Si vs %S, Rho = 0.58, p=0.002. The data were log transformed and the Pearson correlation coefficient for ln Si vs ln %S, r = 0.67, p< 0.0001. There was a significant correlation between first phase insulin (FPI) and BMI r = 0.55, p = 0.003 and a better correlation between FPI and lnSi r = -0.66, p = 0.0002 than between FPI and ln%S r = -0.51, p = 0.0067. **Conclusions:** There was a significant correlation between Si and %S but HOMA was less able to differentiate differences in insulin sensitivity between L and O pregnant women than the minimal model. Differences in FPI in L and O subjects contributes to the minimal model's ability to differentiate differences in insulin sensitivity between L and O subjects.

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HYPERTENSIVE DISORDERS IN TYPE 1 DIABETIC WOMEN DURING PREGNANCY.

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Aims: To evaluate if the type 1 diabetic pregnant women are at increased risk for hypertensive disorders. **Materials and Methods:** Beginning on 1 January 1980 to 31 dicember 1998, clinical data of 276 pregnant women with type 1 diabetes from Diabetes and Pregnancy Center of University of Palermo were collected retrospectively and entered in a computerized data base. Age, duration and complications of diabetes, parity, pre-pregnancy Body Mass Index (BMI), gestational weight gain, glycosylated hemoglobin at first examination. insulin requirement per day at the beginning (IR1) and at the end (IR2) of pregnancy and infant body weight at delivery. The hypertensive disorders in pregnancy were classified according to National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy in 1)Chronic hypertension(CH), 2)Preeclampsia-Eclampsia(PE), 3)PE superimposed on CH and 4)Transient hypertension(TH). Statistical analysis was done using SPSS6.1. **Results:** Nine patients among 276 women (3%) were affected with CH. In the CH patients the duration of diabetes (5.3± 4.3 vs 2.0± 1.1; p< 0.002), the IR1 (24.1± 20.2 vs 10.0 ±14.8; p< 0.001), IR2 (52.6 ±43.0 vs 24.0± 29.0; p< 0.03) were higher respect with normotensive patients. The MANOVA analysis show that CH is influenced by duration of diabetes (F 7.48; p < 0.007), parity (F 5.88; p< 0.01), IR1 (F 9.64; p< 0.002) and IR2 (F 11.4; p< 0.001). Twenty patients (7%) had PE and 7 patients had (2.5%) TH. **Conclusions:** In type 1 diabetic patients the prevalence is similar to that of normal population. The factors that influenced the manifestation of PE are the complications and the duration of diabetes among the type 1 diabetic patients.

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IS 24-HOUR BLOOD PRESSURE SUPERIOR TO CLINIC BLOOD PRESSURE IN PREDICTING PREGNANCY INDUCED HYPERTENSION IN TYPE 1 DIABETES.

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Aim: To determine if 24-hour blood pressure (BP) monitoring in early pregnancy is superior to clinic BP in predicting pregnancy induced hypertension in type 1 diabetes. **Materials and Methods:** During 1996-1997 we recruited 79 pregnant Caucasian women with IDDM admitted to our obstetric clinic prior to 17 weeks' gestation. Women with diabetic nephropathy, anamnestic hypertension or early abortion were excluded. Twenty-four-hour BP monitoring was performed at 10 weeks' and 28 weeks' gestation. Urinary albumin excretion, HbA_{1C} and clinic BP were measured regularly during pregnancy. Pregnancy induced hypertension was defined as two consecutive measurements of diastolic BP >140/90 mmHg. **Results:** Fifteen women developed pregnancy induced hypertension (10 with proteinuria) and 64 did not. At 10 weeks' gestation mean age of the women was 31 (SD 5) vs. 30 (5) years, diabetes duration 16 (11) vs. 13 (14) years, HbA_{1C} 7,3 (0,8) vs. 7,4 (1,0) % and urinary albumin excretion 94 (range 3-752) vs. 16 (1-216) mg/24h. Clinic BP was 126/71 (13/9) vs. 115/69 (11/9) mmHg (p<0,01/NS) compared to 24-hour daytime BP 126/71 (12/7) vs. 119/71 (10/6) mmHg (p<0.05/NS) and the nighttime BP as percentage of daytime BP was 89/83 (5/6) vs. 88/86 (6/7) % (NS/NS). At 28 weeks' gestation clinic BP was 130/75 (15/7) vs. 116/71 (11/10) mmHg (p<0,001/NS) compared to 24-hour daytime BP 143/74 (20/8, n=7) vs. 121/71 (10/7, n=31) mmHg (p<0,001/NS) and the nighttime BP as percentage of daytime BP was 85/87 (11/10, n=7) vs. 89/85 (7/6, n=26) % (NS/NS). The relative risk of developing pregnancy induced hypertension with a systolic BP > 120 mmHg early in pregnancy was 3,2 (clinic BP) vs. 2,1 (24-hour BP). The 24-hour BP monitoring was resource demanding and very unpleasant for the pregnant women. **Conclusion:** Twenty-four-hour BP monitoring in early pregnancy was not superior compared to clinic BP in predicting pregnancy induced hypertension.

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ESTIMATION OF LIPID CHANGES IN TYPE 1 DIABETIC WOMEN.

During normal pregnancy, serum lipid levels increase. Lipid metabolism disorders, related to insulin deficiency are common in type 1 diabetes. The aim of this study was to estimate lipid changes in pregnant women with type 1 diabetes. The subjects studied were 32 patients in average age 27,0±4,9 years, suffering from this disease for mean 9,7±7,1 years, with good control of diabetes (mean HBA1c level during pregnancy =5,8±1,5%) and without macroproteinuria. All diabetic women were divided into two groups: 1.without microangiopathy (n=22) and 2.with micro angiopathy (n=10). The control group consisted of 37 pregnant women aged 25,1±3,8 years with normal glucose tolerance. In successive periods of pregnancy (first trimester - T1, second - T2, third - T3) the concentrations of serum lipids: total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride (TG) and additionally in 12 diabetic women also apolipoproteins ApoA1 and ApoB, were measured. During T2 and T3 the diabetic group had in comparison to T1, significantly elevated level of plasma: TC, HDL, LDL, TG, ApoA1 and ApoB concentrations.

mmol/l	TC	LDL	HDL	TG	ApoA1	ApoB
T-1	4,78±0,93	2,87±0,63	1,33±0,43	2,54±1,19	3,15±0,93	1,53±0,34
T-2	5,88±0,75	3,38±0,66	1,73±0,32	3,84±1,40	3,88±1,02	2,42±0,89
T-3	6,77±1,53	4,09±1,06	1,57±0,47	5,63±1,61	4,17±1,36	3,34±0,45

*p<0,05 "p<0,001 ◊NS. Mean lipid level in respective trimesters in diabetic group did not differ significantly from levels in control group. Mean ApoA1 and ApoB increase level did not correspond with the mean increase level of HDL and LDL. Group 1 during T1 and T2 in comparison to group 2 demonstrated lower level of TG concentration (p<0,05).**Conclusions:** Lipid changes in pregnant women with type 1 diabetes and with good control of diabetes did not differ significantly from the changes observed in normal pregnancy. In diabetic pregnancies with microangiopathy, significant differences in TC levels were not observed, however, this group had significantly TG level as compared with patients without microangiopathy.

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PREGESTATIONAL DIABETES REDUCES MATERNAL RED CELL MEMBRANE DOCOSAHEXAENOIC ACID IN MOTHER AND HER FETUS.

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Docosahexaenoic (DHA) and arachidonic acid (AA) are major structural and functional components of brain, retina and blood vessels. Diabetes impairs the delta-6 desaturase enzyme required for DHA and AA synthesis and as plasma DHA choline phosphoglyceride was reduced in both maternal pregestational diabetic women and their fetuses at term, incorporation of the latter into membranes may also be reduced in the presence of diabetes. **Aim:** to test for deviation in membrane composition in diabetic pregnant women and cord blood. **Method:** maternal and cord blood was collected from pregestational diabetic & control women. After centrifugation the red cells were washed with heparinised saline, the lipid extracted and the choline (CPG) and ethanolamine (EPG) phosphoglycerides separated by gas liquid chromatography. **Results:** There were substantial differences in DHA levels in maternal and cord CPG fractions (table).

Table	Red cell CPG AA %		Red cell CPG DHA%	
	Control	Diabetic	Control	Diabetic
Mothers	11.0±0.36* n=55	9.8±0.56* n=16 .	5.34±0.32¤	3.62±0.31¤
Cord	14.6±0.52† n=38	12.2±0.81† n=29	5.96±0.39#	3.99±0.38#

* & ¤ p=< 0.01, † & # p= < 0.05, ± = SEM.

Only the % DHA EPG concentration was significantly reduced in the maternal red cells of diabetics 5.95±0.51 Vs 7.41±0.30 % p < 0.05. The major differences occurred in the CPG which is in the outer layer of the membrane and consequently in intimate contact with receptor activity. As DHA is cardioprotective and a key structural component of neural tissue, the changes seen could contribute to the neurovascular pathology of diabetes. **Conclusion:** During pregnancy diabetes is associated with disturbance of essential fatty acid metabolism leading to outer membrane distortion and the latter may influence fetal vascular development.

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THE IMPACT OF PREGNANCY ON DIABETIC RETINOPATHY

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The aim of our study was to establish whether pregnancy affects the development and progression of DR in pregnancy. Patients and methods: We studied 48 diabetic pregnant women (38 IDDM, 10 NIDDM, age: 29.4±4.6 yrs) and 20 non pregnant women (19 IDDM, 1 NIDDM, age 32.2±5.4 yrs). Patients underwent ophthalmologist evaluation (photograph of fundus oculi) at I and III trimester of gestation and 6 months postpartum; controls were evaluated at time 0, 6, e 12 months. Retinopathy was classified according to ETDRS criteria. We defined progression or development of retinopathy "de novo", upgrading of DR from early to late pregnancy (grade 0-5) or DR changes requiring additional photocoagulation. Changes were defined whether present in one or both eyes. Results: The progression of DR was showed in 29% of women during pregnancy versus 15% of the control group ($\chi 2$: ns). In the control group, a progression was observed in 3 patients with pre-existing retinopathy. Among pregnant women, 1 patient with pre-existing retinopathy required photocoagulation therapy in late pregnancy, 2 patients showed a progression only in one eye, 9 patients showed retinal changes which persisted also after delivery, whilst 2 patients, worsened in pregnancy, improved at the post partum. The progression of DR was associated to: disease duration (17.8±6.4 vs 11.4±8.2 yrs, t-test: p=0.008), the age of diagnosis (11.8±5.3 vs 16.3±8.5 yrs, t-test: p=0.03), the I trim systolic BP (123.2±7.8 vs 116.9±12.5 mmHg, t-test: p=0.03) and diastolic BP during II trim (74.3±7.2 vs 69.7±7.3 t-test: p=0.05) and III trim (77.4±6.1 vs 72.5±8.5; t-test: p=0.04), higher levels of HbA1c during I trim (7.2±1.5 vs 6.3±1.3% t-test: p=0.04). In the progressive group, 57% of the patients developed a pregnancy induced hypertension compared to 21% of the non progressive group ($\chi 2$: p<0.05). Regulatory mechanisms, controlling microvascular flow, might be impaired in diabetes are furtherly worsened by hypertension.

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PROSPECTIVE POPULATION BASED STUDY OF THE EFFECT OF PREGNANCY ON DIABETIC RETINOPATHY

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Aims: To determine the progression of retinopathy during diabetic pregnancy relative to baseline eye examination. **Methods:** Prospective collection of population based information on pregnancies in women with diabetes who booked with pregnancy within the Northern region of England between 1994-1996. **Results**: 314 women booked with 345 pregnancies between 1994-1996. The eyes were examined in 85% of the women during pregnancy. In 41 %, eyes were dilated and examined at booking and at 28 weeks gestation. Diabetic retinopathy was present before onset in 64/345 pregnancies [18.6 %]. During pregnancy 26 of those with background retinopathy at onset remained stable, but diabetic retinopathy progressed in 17/64 [26.6 %], retinopathy regressed in 12/64 [18.8%]. Of the 17 who developed proliferative retinopathy, 8 received laser treatment. New onset background retinopathy was reported in 34/345 pregnancies [10%], 4 progressed to proliferative retinopathy. In 3/4 laser treatment was required. **Conclusion:** Significant sight threatening new onset diabetic retinopathy can develop during pregnancy. 26.6 % of background retinopathy at onset of pregnancy progresses to sight threatening retinopathy. Regular examination of eyes during pregnancy is essential as retinopathy is present in 23% of diabetic pregnancies and in 3.2% diabetic pregnancies laser treatment is required

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INFLUENCES ON QUALITY OF GLYCAEMIC CONTROL IN TYPE 1 DIABETES

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Aims: To examine modifiable influences on glycaemic control in type 1 diabetes, and assess adverse consequences in terms of hypoglycaemic episodes. **Materials and methods**: A cohort of 1919 patients with type 1 diabetes with questionnaire data on health behaviours and centrally measured HbA1c. **Results**: A clear influence on glycaemic control was the frequency of insulin injection. Mean HbA_{1c} in those whose daily injection frequency was once, twice, 3-4 times, 5 or more times and continuous subcutaneous infusion was 8.6%, 8.9%, 8.5%, 8.4% and 8.1% respectively (p 0.0001 for trend). Mean number of severe hypoglycaemic episodes in the last year were 0.4, 1.3, 1.2, 1.4 and 0.5 (p=0.4 for trend). Non-severe hypoglycaemic episodes in the last month were 4.3, 4.1, 5.5, 5.2 and 8.5 (p=0.0001 for trend). Over 90% of patients self monitored their blood glucose; 54% of these at least once a day. Glycaemic control was better in this latter category compared to those who monitored less often or not at all (8.3% vs , 8.8% and 9.0%, p=0.0001). Severe hypoglycaemic episodes occurred 1.5, 1.0 and 0.8 (p=0.02 for trend) times in the last year in these categories. Glycaemic control was also better in those who used their results to adjust the dose of insulin (8.5% vs 9.0%, p=0.0001), although frequency of severe hypoglycaemic episodes was non-significantly greater (1.3 vs 0.8 in the last year, p=0.1). Control was better in those who were members of a diabetes organisation (8.4% vs 8.6%, p=0.004), and in those who had attended a diabetes education session in the last year (8.3% vs 8.6%, p=0.01). **Conclusions:** Increasing the frequency of insulin injections, encouraging regular self monitoring and responsive adjustment of insulin dose, regular teaching sessions and membership of a diabetes organisation all serve to improve glycaemic control, but with some adverse consequence in terms of hypoglycaemic episodes. This information should be used to inform patients of risks and benefits of improved glycaemic control, and allow them to decide how tight their glycaemic control should be.

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LONG-TERM COMPLICATIONS OF TYPE 2 DIABETES: PRELIMINARY RESULTS FROM A NEW ECONOMIC MODEL

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Aims: The worldwide economic burden of type 2 diabetes is significant and is rising rapidly due to a number of demographic and lifestyle factors (eg increasing obesity, an increasingly elderly population and decreasing physical activity). The major contribution to the high economic cost to society comes from the chronic long-term complications of type 2 diabetes. We have developed a long-term economic model to assess the effects of treatment on long-term complications and their associated costs.

Methods: Our model is based on an earlier NIH model and uses a Markov process whereby patients in the model move through a succession of modules according to a variety of transition and event rates, so that their condition and life history is regularly updated until death. The rates vary by duration of diagnosis, age, gender and ethnicity. Separate modules estimate micro- and macrovascular complications and overall mortality. Microvascular complications modelled include nephropathy, neuropathy and retinopathy; the model includes coronary heart disease and stroke as macrovascular complications. Costs for the UK were taken from the Cardiff database (hospital, clinic, primary care and community costs) and from published sources (prescription rates, drug costs). UK disease progression data (including UKPDS) were incorporated.

Results: In general, spending on all elements of health care is substantially increased for patients with type 2 diabetes. Hospital admissions and average length of stay are both increased and attendance at hospital clinics is more frequent. Patients with type 2 diabetes visit their primary care physician three to four times more frequently than other patients. The overall cost per patient for patients with type 2 diabetes under 70 years is over twice as great as for those without the disease.

Conclusions: We have used a new long-term cost-effectiveness model to assess the economic burden of type 2 diabetes on the health care system in the UK; the incorporation of appropriate data will allow its use in other countries. The model can also be used to determine the effects of risk factor modification and to compare the cost-effectiveness of alternative therapeutic strategies.

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Stability and feasibility of using a fingerprick blood spot HbA1c% assay versus standard venous sampling

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Aim: To assess the stability and feasibility of using a fingerprick blood sample to analyse HbA1c. **Materials and Methods:** In 50 patients samples were collected from a fingerprick directly onto filter paper (20µl) and compared with a simultaneous venous EDTA sample (2ml). HbA1c% was analysed from the elutant by HPLC on day 0, 3, 7 and day 14 and compared to venous HbA1c% on day 0. **Results:** The statistical method used was a modified Delta analysis. There was an excellent correlation ($p<0.01$) between the venous and filter paper HbA1c% at day 0 ($r = 0.968$), day 3 ($r = 0.952$), day 7 ($r = 0.962$) and at day 14 ($r = 0.891$). The mean difference was -0.38 (95% CI -0.52 to -0.25), at day 0, -0.42 (95% CI -0.59 to -0.2 6) at day 3, -0.50 (95% CI -0.67 to -0.34) at day 7 and -0.13 (95% CI -0.38 to +0.10) at day 14. The mean percentage difference on days 0, 3, 7 and 14 was 4.46%, 4.82%, 5.55% and 9.04% respectively. Of the 55 postal samples the average difference was -0.25 (95% CI -0.41 to - 0.08).The coefficient of variation for the two assays was 3%.**Conclusions:** Postal HbA1c % assessment by filter paper provides a reliable, convenient, inexpensive and patient friendly method which avoids the need for pre-clinic venous sampling. It is particularly useful when close and frequent monitoring is needed. This assay needs to become more widely available and will help achieve optimal glycaemic control.

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DO WOMEN WITH TYPE 2 DIABETES REQUIRE MORE INTENSIVE CARE FOR THEIR DIABETES THAN MEN?

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Aims: To investigate whether sex of the patient affects the outcome of newly diagnosed type 2 diabetes. **Methods:** All the cases (363) of newly diagnosed type 2 diabetes presenting in one year from 185,000 people were identified. Patients were diagnosed through normal health care processes and no active screening occurred. Glycaemic control, weight, blood pressure and lipids were measured. **Results:** Three hundred patients (83%) attended the diabetes education programme. Body Mass Index was significantly higher in women than men at diagnosis, mean 31.0 kg/m^2 versus 28.9 kg/m^2, $p=0.01$. Three months after diagnosis, there was significant weight loss of -1.1kg (95% CI -1.5 to -0.6, $p<0.001$). Women and men lost a similar amount of weight. HbA1c was not significantly different between men and women at diagnosis. Glycaemic control improved, for all patients mean HbA1c fell from 10.3% at diagnosis to 8.1% at 3 months. At review, women had significantly higher systolic blood pressure than men, 142.8 mm Hg compared to 136.4 mm Hg (mean difference 6.4 mm Hg 95% CI 2.0 to 10.8, $p=0.004$). Diastolic blood pressure was not significantly different. At 3 months, total cholesterol levels, but not triglyceride, were significantly higher in women, mean 6.1 mmol/l compared to 5.5 mmol/l, mean difference 0.6 mmol/l (95% CI 0.3 to 0.9, $p<0.001$). At one year, glycaemic control remained inadequate with 77% of women and 64% of men with HbA1c > 7% (NR 4.1-6.5). **Conclusion:** Management of type 2 diabetes in the first year failed to achieve good glycaemic control and women fared worse than men.

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TeleDiab – A Telemedicine approach to Diabetic Care

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Aims – To assess the feasibility of using PC based video teleconferencing (PCVT) technology to facilitate diabetic retinopathy screening.

Materials and Methods –PCVT was used to enabled secondary care (hospitals) and primary care (general practices) to share patient management. Hypothetical patient data was used consisting of colour retinal images (normal, non diabetic eye disease and diabetic retinopathy of varying severity) and a structured management questionnaire. This data was jointly discussed using PCVT between clinicians. Equipment consisted of entry level MS WIN95 PCs fitted with video conferencing card and telephone handset. Communications were via ISDN2 telephone lines. Software consisted of MS NetMeeting, PCCTurbo (Olsy) and a database developed in-house using MS Visual Basic. The system provided the facility to transfer files and share database and imaging software. Included in the software were tools to assist with the discussion of features of retinal images which allowed for example, lesions to be selected and annotated. Seven General Practices were recruited and provided with equipment and training in the use of PCVT. Effectiveness was assessed by comparing GPs ability to grade a standard set of retinal images prior to and after a PCVT session. User activities during PCVT sessions were recorded.

Results – (1) Response by all GPs to the clinical use of PCVT was positive (2) Clinicians' confidence in the operation of PCVT improved with use; (3) PCVT sessions lasting longer than 45 minutes resulted in user fatigue; (4) The mean duration for discussion of each patients management was initially 6 minutes 8 seconds (Std.Dev.=2:1, n=28) but later reduced to 5 minutes 2 seconds (St.Dev.=1:4, n=20) as the user became experienced with the system.. (5) The preferred tools used to aid discussion of retinal images were: (a) text annotation; (b) pointer; (c) ellipses; (d) zoom.

Conclusions –.Two major factors which influenced effectiveness were: (1) Clinical - the greater the clinical knowledge of retinopathy the more effective the PCVT session; (2) Computer literacy - the higher the competence with MS Windows the more effective the session. Use of PCVT has the potential to provide a contribution to the delivery of education for health care professionals in diabetes care. Use of PCVT can facilitate communications between primary and secondary care so that diabetic retinopathy screening may be efficiently managed and co-ordinated.

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CAN THE TARGETS FOR BLOOD PRESSURE CONTROL IN THE UNITED KINGDOM PROSPECTIVE STUDY BE ACHIEVED IN ROUTINE CARE?

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Aims: Intensive blood pressure (BP) control in the United Kingdom Prospective Diabetes Study (UKPDS) achieved a mean BP of 144/82mmHg with dramatic improvements in clinical end points. Audits of care have suggested that routine physician intervention results in poor blood pressure control. We have assessed whether a protocol guided, nurse practitioner, blood pressure management system could achieve better results in BP control. **Materials and Methods**: Patients were recruited from routine diabetes clinics with a BP of >145/85 and seen monthly with blood pressures measured in the sitting position, by an automatic machine, the Omron 705-CP. Three BP readings were taken, the mean of the second and third readings recorded. Stepwise introduction of a thiazide diuretic, ACE inhibitor and alpha blocker in patients without major morbidity was supervised by a nurse practitioner. **Results**: To date 95 patients have entered the clinic: 10 Type 1 and 85 Type 2; age 64 ± 9 years (mean±SD); body mass index 29±4.4. Initial mean systolic BP was 166±18.7 and diastolic 90±14.6. At visit 2 equivalent readings were 163±18.8 and 92±13.5; visit 3 150±16.9 and 84±12.6; visit 4 149±13.5 and 80± 9.4; visit 5 143±12.6 and 81± 2.7. Mild side effects caused medication changes in 10 patients. **Conclusions**: Results to date suggest nurse practitioner management of hypertension in diabetic patients is both safe and effective.

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MEETING THE CHALLENGE OF IMPLEMENTING DIABETES STANDARDS OF CARE

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Aims: The majority of diabetes care is delivered in the primary care setting. Studies reveal large gaps between recommended standards of diabetes care and current practices in these settings. We have developed an innovative team care, health management approach to diabetes care using an internet-based computer program to facilitate diabetes standard adoption by PCPs in a large HMO. **Materials and Methods:** The program began with providers generating consensus on standards for metabolic control, screening for eye, foot and renal complications, hyperlipidemia and hypertension. Best practice provider and patient education was included in the program. Patients are screened and stratified according to risk and interventions are reported by software designed for this 12-month study. A unique feature of this program is a "tracker", who assures that recommendations are carried out and who follows the patients in the program. We have enrolled over 300 patients followed by over 20 PCPs at two sites. **Results:** Given our primary hypothesis to reduce mean HbA1c by at least 0.5%, our six-month data has been significant.

Accountability Measure	Baseline	6-month
Patients receiving ≥1 HbA1c test/year	79%	100%
Patients with the highest risk HbA1c level (>9.5%)	20%	15%
Patients receiving a lipid profile once in 2 years	66%	100%
Patients with blood pressure <140/90mmHg	30%	53%

Conclusions: Providers feel secure that routine tasks are not being overlooked and that they can spend more time dealing with current and preventive medical issues. Patients feel more knowledgeable and more able to take on the responsibility of their care. In addition to improving the ability of the clinic personnel to comply with standards that they have agreed are vital to good clinical care, the program provides a record of the care given enabling HMO management to document adherence to the standards required by various accrediting agencies (e.g. HEDIS, Facct, DQIP).

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COMBINED CONTROL OF INSULIN TREATED PATIENTS IN GENERAL PRACTICE AND A DIABETES CLINIC. A QUALITY ASSURANCE STUDY.

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Aims. Insulin treated patients have in our country traditionally been controlled in specialized diabetes clinics (DC). Due to the increasing number of insulin treated patients, beyond the capacity of many clinics, we wanted to test the quality of combined control in general practice (GP) and DC.

Materials and Methods. About 90 % of 71 practitioners (P) within a region of 125000 inhab.and a range of 60 km from DC, paticipated. They should control of their own patients each 3 months. All patients were seen at DC once a year. The Ps and the diabetes team met before start of the study and then once a year to coordinate clinical guidelines. Lab. values were exchanged electronically. The results from DC were sent to GP with recommandations. The patients had a small record for use both at DC, GP and at home. The study was designed for 2 years and the values compared with values from the control at DC the preceeding 2 years. Only insulin treated patients without major problems were included.They could contact DC at all times.

Results. 73 insulin treated patients were included and the results of 50 patients passing the first year control at DC before april 99 are reported. 3 patients left the study and returned to DC, one patient died at home, cause of death unknown. Of the remaining 46 patients, 23 were already treated with antihypertensive drugs.The diabetes duration was 19 years (3-71) mean age 58 years (28-80) . All except one patient had normal creatinine levels. Albumin/creatinine ratios were measured: 2.5 - 5 named borderline-, values > 25 named macro-. The ratios at start of study and at one year showed: normals 26/21, borderline 1/7, micro- 6/8, macroalbuminuria 13/10. HbA1c values (mean) were 8.5 and 8.2 the preceeding two years at DC. 8.4 at start of the study and 8.0 measured in GP each 3 months and 8.1 at one year in DC (NS). Almost all patients wanted to continue the combined control after ending the study.

Conclusion The quality of combined control was equal to that of DC alone.Control of albumin excretion and blood pressure seems to be improved by combined control.

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THE USE OF OUTCOME RESEARCH TO ASSURE DIABETES TREATMENT QUALITY.

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Aim. Intensified metabolic control, careful monitoring of late diabetic complications and intervention against cardiovascular risk factors improve the prognosis of IDDM and NIDDM patients. Cost-benefit analyses have shown that intervention and intensified care in diabetic patients are at least as favourable as in the non-diabetic population. In the coming years the challenge in the treatment of diabetes will be to apply these results into the general diabetes population, and the aim of the present study was to analyse the treatment quality in a routine outpatient clinic.

Methods. We have investigated outcome data in 2011 IDDM-patients living in Copenhagen County and followed at Steno Diabetes Center in the period 1995-1997. The mean age and diabetes duration were 49 years and 20 years, respectively, and 54% of the patients were males.

Results. HbA1c was measured at least once yearly in more than 99.5% of the patients and the mean annual number of measurements were 3 in each patient. Urinary albumin excretion was measured in 87.0%, 87.1% and 80.5%, and blood pressure in 71.4%, 76.7% and 79.8% in the years 1995, 1996 and 1997, respectively. Foot-inspection was performed in 41.1%, 46.1% and 44.8% of the patients. The mean HbA1c (normal range 4.1-6.4%) were 8.8%, 8.7% and 8.6%, respectively. The frequency of patients with an HbA1c-value below 140% of the mean value in non-diabetics (< 7.5%) were 14.6% in 1995, 15.4% in 1996 and 17.7% in 1997.

Conclusion. In unselected IDDM-patients followed at the outpatient clinic at Steno Diabetes Center, some but not all important clinical variables are measured with equally high frequency. Further analyses may help to explain, why there is such a difference, and hopefully, to explain how the tendency to improved metabolic control can be further extended.

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THE DIAS COMPUTER MODEL UNCOVERS FREQUENT INSULIN "FAILURE" IN POORLY CONTROLLED TYPE 1 DIABETES.

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Aim: To evaluate the accuracy of blood glucose modelling by the Diabetes Advisory System (DIAS). **Materials and Methods:** DIAS is a decision-support program for Type 1 diabetes, representing glucose and insulin metabolism by a compartmental model. Patients record pre-prandial and bedtime blood glucose (BG), insulin doses, and meal carbohydrate over 4 consecutive days. From these data the program generates a simulated continuous BG profile. We analysed simulations from 20 patients (12 male) seen in out-patients with unexplained high blood sugars: mean (SD) HbA_{1c} 10.4(1.8)%, age 37(11)yrs, diabetes duration 15(8)yrs, and insulin dose 0.8(0.2)U/kg/d. 19 patients were on basal-bolus regimens (4 using Lispro), and one on twice-daily pre-mixed insulin. Dietary collection and analysis were supervised by a dietitian. **Results:** Recorded BG values ranged from 2.0 to 28.9mmol/l, and only one insulin dose was declared as omitted by a patient. The mean discrepancy between simulated and observed BG (D) ranged from 1.7 to 6.5mmol/l per patient, with an average (for all 310 BG results) of 3.7(3.2)mmol/l. In correlation analysis (simulated vs observed BG), r = 0.50 (P<0.001). In 16 patients we found that selective removal of insulin doses (median 3, range 1 to 6 doses over 4 days) considerably improved the fit of the DIAS simulation; in an extreme case the patient on pre-mixed insulin appeared to have received only 3 of 8 doses. Only 3 patients had obvious injection site abnormalities. These "omissions" in the 16 patients significantly improved the accuracy of the modelling of BG values, not only above 10mmol/l (D=3.2 vs 5.2mmol/l, P<0.001) but also across the whole BG range (r = 0.70 vs 0.37, P<0.001). The average discrepancy for all 20 patients fell to 2.7(2.6)mmol/l (P<0.001). **Conclusions:** Blood glucose modelling using DIAS indicates a high rate of insulin "failure" in poorly controlled Type 1 diabetes, which may result from omission of injections or non-absorption of insulin. This computer-based analysis may improve detection of such problems in clinical practice.

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STAGED DIABETES MANAGEMENT®: IMPLEMENTATION IN GERMANY

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Aim: Diabetes mellitus is a frequent chronic disease which needs stage adjusted diagnostic and therapeutic interventions. The St. Vincent's-Declaration has set the goals of these interventions on an population-based approach. Therefore these procedures should be made available for primary health care providers. SDM stresses attention to timelines and glycemic targets, with rapid modification of therapies until an effective therapy stage is achieved. The actual german SDM flow charts establish individual therapy goals in relationship to the age of the patient with a split for geriatric and non-geriatric patients. **Materials and Methods:** We have evaluated the effects of the introduction of Staged Diabetes Management in Hamburg, Germany. From a certain time point on 370 patients with diabetes mellitus (type 1 and 2) where enrolled in 12 GP offices (SDM n=196, control n=174). The randomisation was performed per centre. Data collection was done at the day of inclusion and after 3 and 6 months. **Results:** During the study period the HbA1c showed a significant decrease in both, the SDM- (0,66) and the control-group (0,45), (7,62 vs. 6,98% and 7,26 vs. 6,81%; p<0,001). In the SDM-group the greatest decrease (-0,80) can be shown in the younger (< 65 y.) type 2-patients (7,47 vs. 6,67%; p<0,0001). Contrary in the control-group the greatest decrease (-0,54) was found in the group of the elder (> 65 y.) type 2-patients (7,17 vs. 6,63%; p<0,001). The occurrence of severe hypoglycemic episodes in the elderly type 2-patients was 0,06/patient/year in the SDM-Group and 0,16/patient/year in the control-group. The number of self-glucose-monitoring of type 2-patients increased in the SDM-group from 0,41 to 0,91 tests per day. In the control-group there was no significant change in the frequency of self-glucose-monitoring (0,55 to 0,57 tests per day). As an important second-line effect the satisfaction of therapy (-12 vs. +43) and the quality of life (-6 vs. +12) increased during 6 months in type 2-patients treated with help of the SDM-guidelines (both measured with the Bradley questionnaire). **Conclusions:** This controlled study revealed a significant improvement of glycemic control, an increase in the safety of therapy and the quality of life especially in type 2-patients by the introduction of SDM in the primary health care setting.

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Staged Diabetes Management: Improvements in Health Status and Patient Self-Management Skills Among Patients in a Managed Care Setting. L. Blonde[1], R. Guthrie[1], T. O'Brien[2], M. Testa[3], R. Zimmerman[1], M Sandberg[1], A. Rein[4], M. Wargo[4], and B. Ginsberg[2]. [1] The Ochsner Clinic, New Orleans, LA, USA; [2] Becton Dickinson and Company, Franklin Lakes, NJ, USA; [3] Harvard School of Public Health, Boston, MA, USA; [4] Covance Health Economics and Outcomes Services, Washington, DC, USA.

Staged Diabetes Management™ (SDM) provides comprehensive practice guidelines designed to improve diabetes care and is usually focused on primary care physicians. We compared the one-year outcomes of 93 patients with Type 2 diabetes managed by diabetes nurse educators using SDM with those of 73 patients receiving usual care (UC) from their general internists. Mean baseline HbA1c values were 8.58% in the SDM and 8.34% in the UC group (NS). The SDM patients experienced greater improvements in glycemic control than the UC group. After 12 months, the mean change in HbA1c was -1.13% in the SDM group and -0.47% in the UC group (p<.005).

A survey instrument completed by patients indicated that the SDM patients were more knowledgeable about diabetes than the UC group and demonstrated greater adherence to self-management activities. After 12 months, 86% of the SDM patients but only 49% of the UC patients knew the definition of HbA1c (p<.02). Fifty-eight percent of SDM patients measured their blood glucose levels at least twice daily compared to 27% of the UC patients (p<.001). Daily foot examinations were performed by 77% of the SDM patients compared to 58% of the UC patients.

Resource utilization data were collected for the SDM patients. Over 12 months, the mean amount of face-to-face time spent by nurse educators was 240 minutes per patient with an additional 126 minutes per patient of telephone contact. During the first three months, the mean amount of face-to-face contact was 42 minutes while the mean amount of telephone contact was 50 minutes. During the final three months there were 40 minutes of face-to-face contact and 28 minutes of telephone contact, demonstrating decreased resource utilization requirements as glycemic control improved and patients learned more about their diabetes. Additional analyses including physician visits, ER visits, and hospitalizations are being performed.

This study demonstrated that a team of diabetes nurse educator care managers, endocrinologists and primary care physicians using SDM can improve glycemic control, patients' knowledge of diabetes and self management activities.

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COMPATIBILITY AND IMPLEMENTATION OF DIABETES QUALITY MANAGEMENT SYSTEMS IN GERMANY

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Aims: The capability of seven diabetes quality management systems (DPV 4.0, DiabCare Data 1.5, mediNET QM 1.69, FQSD 1.12, Diqual 2, Q-Med Diabetes ß, DiabCare Fax System) to aggregate and analyse structured diabetes data sets was investigated. Particulary the ability to contribute data to a national diabetes register and to allow center-external benchmarking was examined. **Materials and Methods:** The software-producers were asked to specify their systems by structured questionnaires and to supply the latest versions of their software. Then the records of 20 diabetes patients of Klinikum Innenstadt were entered into the systems. According to ISO/IEC 9126 a metric was established to test each system with respect to functionality, robustness, useability, efficiency, changeability and interoperability. The DiabCare Basic Information Sheet for adults (BIS) served as standardized data set of European consensus. The percentage of matched BIS-items was counted for each system. BIS-compliance and the way of data-interchange were taken as measures of the possible integration into an overregional quality-network. **Results:** According to ISO/IEC 9126 a great diversity between the seven systems has been shown. The following table summarizes the number of implementations at GPs, at hospitals, the percentage of BIS-compliance and the number of central servers of the tested systems.

	DPV	DC 1.5	MediNET	FQSD	Diqual	Q-Med	DC Fax
GPs	300	104	100	150	122	122	104
Hospitals	150	37	200	50	148	148	37
BIS-items	92%	100%	100%	100%	51%	unknown	100%
Servers	1	1	2	1	1	1	1

Conclusions: 1091 institutions in Germany can aggregate data with over 90% BIS-compliance. Secure internet communication, standardized quality indicators and a unique code for the specification of participants are necessary for the transmission to central servers and a prerequisite for the realisation of a national diabetes register.

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THE PROSIT-PROJECT: A QUALITY MANAGEMENT SYSTEM FOR PRIMARY DIABETES CARE IN GERMANY

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Aim: The Proteinuria Screening and Intervention project started in three German states in order to improve diabetes care in patients with microalbuminuria (MAU). **Methods:** In this project general practitioners (28%) and diabetes specialists (72%) screen for MAU and enroll the positive tested patients into an intervention programme. The structured documentation of all patients is sent to a central server, processed by using optical mark sense recognition scanners and an automatic evaluation is generated with individual treatment recommendation for each patient. Every three months the central office sends data reports and recommendations to each project participant. Evaluation of aggregated data helps to assist local and regional quality initiatives. **Results:** Currently 2,838 diabetics from 143 practices were evaluated at least once within the PROSIT-project. Counted from the baseline they are in different stages of the project. Results of 674 diabetics with four PROSIT-evaluations (baseline and three quarterly follow-ups as of February 1999, x ± SD) from 92 practices in the PROSIT-project are presented: **HbA1c:** 7.3 ± 1.4% (initially 7.7 ± 1.7 %, p<0.001). 16% of patients with HbA1c > 8.5% (initially 27%, p<0.001). **Systolic blood pressure:** 144 ± 18 mmHg (initially 147 ± 20 mmHg, p=0.001). **Diastolic blood pressure:** 81 ± 10 mmHg (initially 84 ± 11.0 mmHg, p<0.001). 55% of patients reach blood pressure values ≤ 140/90 mmHg (initially 47%, p<0.001). **Total cholesterol** (fasting): 216 ± 42 mg/dl (initially 225 ± 46 mg/dl, p<0.001). **Triglycerides** (fasting): 164 ± 104 mg/dl (initially 185 ± 128 mg/dl, p<0.001). **MAU:** No significant changes were noted for the development of average MAU both in quantitative or semi-quantitative measurements. Micral-Test II (n=470): 24% of patients improved in at least one colour block, 27% worsened, 49% remained stable. No changes in **smoking** habits were reached (10% vs initially 11% smoking). **Conclusion:** Although the PROSIT-project is not a clinical study but the implementation of scientific knowledge into routine patient care, the data show relevant improvements in diabetes care even after this short intervention period. The project is continued under the auspices of the WHO/IDF St. Vincent Action Plan.

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MANAGEMENT OF TYPE 2 DIABETES IN GENERAL PRACTICE: A RISK FOR THE PATIENTS' HEALTH?

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Aims: Improved glycemic control and blood pressure reduce the incidence of diabetic complications. Management of type 2 diabetes mellitus beyond specialized units is mostly insufficient. We screened patients with type 2 diabetes seen for the first time in our diabetes care unit with regard to diabetes duration and therapy, glycemic and blood pressure control and manifest diabetic complications. **Methods**: 50 patients (23 m, 27 f, age 61,3±11,3 years, diabetes duration 8,2±8,1 years) prior treated by a GP were included in the order of their arrival in our unit. Beside intensive interrogation and physical examination, detailed blood and urine analyses including HbA1c and nephropathy screening were performed. 20 patients had a follow up after a mean of 5 months. **Results**: Mean HbA1c levels were 8,6±2,1 %. 46% had not attended a diabetes education program. In patients with HbA1c> 7% (80%, n=40) only 34% did a selfmonitoring of blood glucose levels, 70% had no diabetes diet, 42,5% were not treated with OADs. 8 patients had insulin treatment, 4 with none or inadequate selfmonitoring. 28% of all patients had arterial hypertension. At the time of presentation 44% had systolic blood pressure > 150 mmHg, 18% > 170 mmHg. We found a manifest diabetic nephropathy defined as a microalbuminuria (> 20mg/l urine) in 56% only 21,4% had adequate treatment. 24 % had clinical symptoms of neuropathy, 16% of periphery arterial occlusive disease, 16% missing foot pulses, 50% had reduced vibration threshold. In 8 patients diabetic retinopathy was known. At the time of follow up, all 20 patients had attended our diabetes education program and did adequate self-monitoring. HbA1c-levels had improved significantly to 7,4±1,4% (initially 9,3±2,7%, p=0,001). Systolic blood pressure (144±14,5 mmHg vs. 153±24,9 mmHg) and microalbuminuria (15,3±35,5mg/dl vs. 10,7±23,6 mg/dl) had also improved even though not significantly. **Conclusion**: Management of Typ 2 diabetes by non specialized GPs is even in a country with a good health care system mostly unsatisfactory. Diabetic complications are frequently manifest when patients present in specialized diabetic care units. When treated by specialists the vascular risk profile can be improved quickly. Patients should therefore present earlier in specialized care units. Further training in diabetes care for GPs is necessary and an interdisciplinary care is absolutely required.

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LONG-TERM EFFECTIVENESS OF A QUALITY SYSTEM ON GLYCAEMIC CONTROL AND CARDIO-VASCULAR RISK PROFILE FOR PATIENTS WITH TYPE 2 DIABETES IN GENERAL PRACTICE.

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Aim: Several studies have reported positive effects of quality systems. However, these studies often concern a short follow-up period. This study was conducted to assess the long-term effectiveness of a quality management system on glycaemic control and cardiovascular risk profile of patients with type 2 diabetes treated in general practice.
Materials and Methods: A non-randomized trial was carried out in which 384 GP-treated patients in the intervention, and 85 patients in the control group were followed for 3½ years. In the intervention group guidelines for the care for patients with type 2 diabetes were implemented in two regions of the Netherlands. Implementation of the guidelines was supported by: post-graduate education, peer review, feedback and evaluation of provided care. After the baseline measurement in 1993, patients were measured after 1½, 2½ and 3½ years of follow-up. Data on glycaemic control (fasting blood glucose, HbA1c) and cardiovascular risk profile (blood pressure, BMI, cholesterol, HDL-cholesterol, triglycerides) were assessed. To analyse the data, linear generalized estimating equations were used. Moreover among the patients who underwent all four measurements the change in percentage of patients with an acceptable/good control was studied.
Results: The quality system had a positive effect on the level of HbA1c (β=-0 .25 [0.51;0.00]) after correcting for sex, region, age, level of education, duration of diabetes, mode of treatment and the baseline measurement. After an initial increase of the percentage of patients with an acceptable/good HbA1c (<=7.5%) from 56.8% to 73.6%, the effect diminished to a percentage of 65.5% after 2½ years and increased again to 70.3% after 3½ years. In the reference group the percentage of patients with acceptable/good HbA1c declined from 87.8% to 50.0% after 3½ years. There was no effect on fasting blood glucose and on the cardiovascular risk profile.
Conclusion: It can be concluded that the quality system has a long-term favourable effect on glycaemic control of patients with type 2 diabetes treated in general practice. A positive long-term effect on cardiovascular risk profile could not be demonstrated.

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METABOLIC CONTROL OF TYPE 2 DIABETIC PATIENTS REGISTERED WITHIN PRIMARY HEALTH CARE IN HUNGARY.

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Aims: To obtain hard data about the overall metabolic control of type 2 diabetic patients with oral antidiabetic treatment, a HbA_{1c} mass-screening was performed in Hungary. **Materials and Methods:** Clinical characteristics were recorded and HbA_{1c} values were measured by affinity chromatography (normal range: 4.4 – 6.4 %, interlaboratory coefficients of variance 2.1 – 3.9 %) in 10145 type 2 diabetic patients (age ≤75 years; 41.6 % men, 58.4 % women) with oral antidiabetic drugs (duration of treatment ≥3 years). All the diabetic patients were in the care of GPs (n=978) and the screening procedure was conducted by diabetes centres (n=26) nation-wide. **Results:** The clinical characteristics of the screened diabetic populations were as follows [mean values (5.0-95.0 % CI)]: age 62.2 (46.0-74.0) years; duration of diabetes 10.3 (4.0-21.0) years, duration of oral treatment 9.4 (3.0-20.0) years, BMI 30.2 (22.9-39.0) kg/m^2, waist-hip ratio 0.93 (0.81-1.05). In 46.3 % of patients HbA_{1c} values (≥7.5 %) referred to fair or poor metabolic control. Apart from dietetic instructions and modification of current oral antidiabetic treatment (n=3590), insulin therapy (bedtime with sulphonylureas or insulin alone) was initiated after the screening in 2186 patients. **Conclusion:** In order to achieve and maintain good metabolic control, more appropriate treatment with adequate patient education should be provided for type 2 diabetic patients registered within the primary health care system in Hungary.

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QUALITY OF DIABETES CARE IN A GENERAL PRACTICE NETWORK

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By implementing methods of quality management in Germany there is good knowledge on quality of care of diabetes type 1. But no pupulation based data are availably concerning type 2 diabetic patients. **Aim:** Assessment of quality of care and the participation rate in structured diabetes education of all patients with diabetes as the baseline of an appropriate intervention strategy. **Methods:** From 10/1998 to 3/1999 a diabetes network was created between 15 GP's and the regional diabetes centre. Data on all patients with diabetes were collected with PC-Software DIQUAL3.0. HbA1c (last 3 months) is expressed as relative HbA1c (Original value/mean mean normal of the regional method). **Results** (intermediate, March 29th 1999): 733 diabetic patients (age 67.7y, duration 10.1y) were assessed in 9 practices (45-114 pat/GP). Complications: Blind 0.3%, lower limb amputation 1.2%, diabetic foot ulcer 5%, raised serum creatinin 10.1. 32% of patients participated in a education programme: type 2 patients without insulin: 18.1%, type 2 patients with insulin therapy 62.1%, type 1 patients: 100%. HbA1c values were available in 82.1% of all patients. Relative HbA1c was 1.36 in type 2 patients without insulin therapy, 1.52 in type 2 patients with insulin therapy and 1.57 in patients with type 1 diabetes (multiply relative Hba1c with the mean normal of your method to compare the results!). Fundoscopy (last 12 month) was performed in 59.9%. BP was measured (last 3 month) in 98.4%. Mean BP (syst/diast) was 133.34/78.95 in patients without and 147.50/83.01mmHg in patients with antihypertensive medication (BP<=140 and <90mmHg 35.9%). Only 3.4% of patients were in shared care with a diabetologist. **Conclusion:** Diabetes care in the general practice using a regional network is much better than expected. The majority of typ 2 diabetic patients without insulin therapy reach the HbA1c goal of the UKPDS. Hypertension care should be improved. There is a urgent need for a structured hypertension programme in general practice.

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EFFECT ON GLYCAEMIC CONTROL OF FOUR YEARS SHARING CARE BETWEEN GENERAL PRACTITIONER AND ENDOCRINOLOGIST

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Aims: Optimal glycaemic control is a major goal in the treatment of patients with type 2 diabetes. Many patients are seen by general practitioners (GPs). Guidelines have been issued to support GPs. To help GPs implementing these guidelines, a collaboration scheme between a hospital-based endocrinologist and GPs has been designed in which GPs receive repetitive advice without the need for referral of patients, making the scheme more widely applicable: patients are seen by the GP only.

Material and methods: Annual HbA1c levels of 4 years (0 to 4) were analysed using ANOVA for repeated measurements (R) or Studnt t-test (T) where appropriate (uni- or bidirectional change during study). The group was subdivided according to initial control: adequate (AC: HbA1c≤7%) or inadequate (IC: HbA1c >7%) and to new (N: ≤ 1 year) or longstanding (L:>1 year) diabetes. Change in percentage using medication were compared between 0 and 4 using Chi^2-test.

Results:A total of 263 patients were evaluated : 163 females (60.6%); mean age 65.3 years (range 33-87), median duration of disease: 2.4 years (range 0.1-33.4). HbA1c in the total group fell from 7.25±2.26 to 6.32±1.54 (1; T: p<0.001), rising to 7.00±1.59 (4; R: p<0.001). In 55 patients with IC+N: HbA1c fell from 9.75±2.00 to 6.17±1.33 (1; T: p<0.001), rising to 6.77±1.33 (4; R: p<0.001). In 63 patients with IC+L, HbA1c fell from 8.90±1.53 to 7.23±1.74 (1; T: p<0.001), rising to 7.81±1.66 (4; R: p<0.001). In both AC groups, HbA1c steadily rose: N (43 patients): 5.52±0.78 to 6.75±1.25 (R: p<0.001) and L (108 patients): 5.71±0.91 to 6.74±1.65 (R: p<0.001). Use of sulphonylurea increased from 63.6 to 77.7%, biguanides from 13.4 to 24.5%, both p<0.001; initially no patients were on insulin; 5.2% started with insulin.

Conclusion: Long-term collaboration between GPs and endocrinologist without referral leads to early improvement glycaemic control in patients with inadequate control followed by a gradual rise as seen in patients with intially good control. This steady rise may reflect the natural evolution of the disease.

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TARDIS: A SURVEY MEASURING THE PREVIOUSLY UNDER-ESTIMATED ECONOMIC BURDEN OF TYPE 2 DIABETES IN THE UK

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Aims: The UK Government aims to modernise health and social services. There is an increasing focus on the patient and carer perspective. The TARDIS (**T**ype 2 Diabetes: **A**ccounting for a Major **R**esource **D**emand **I**n Society in UK) survey will provide a basis for future allocation of health and social services resources. It will measure the economic and quality of life impact of type 2 diabetes on patients and their carers. The focus is *the person* with diabetes rather than the cost of diabetes *per se*. **Material and Methods**: TARDIS is a cross-sectional postal survey of a random sample of 1500 people with type 2 diabetes (and their carers) using population-based diabetes registers in four UK centres. The survey examines direct resource use in primary and secondary care, indirect costs, treatment satisfaction, health status and carer burden with validated instruments. The survey questionnaire was piloted in the centres. **Results**: Experiences and outcomes from TARDIS will be reviewed. The design and logistics of the survey, value of the pilots, instruments to capture direct and indirect costs and quality of life data, initiatives to maximise response, mechanisms to explore the extent of responder bias and relevance of comparison of TARDIS results with normative data will be discussed. **Conclusions**: The impact of type 2 diabetes in the UK is significant yet most likely underestimated. The new primary care groups need to manage the total costs of the person with diabetes and understand the patient viewpoint in delivering services and achieving desired treatment outcomes. TARDIS is a timely survey that will allow a better understanding of how the overall economic burden of type 2 diabetes is composed in the UK with its focus on understanding primary care costs as well as other direct costs, but also indirect and intangible costs for patient and carer.

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Metabolic control did not deteriorate between one and three years after the initiation of insulin therapy in type 2 diabetic patients – importance of a structured teaching and treatment programme

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We already published a prospective controlled study evaluating the effects of the same structured education programme one year after the initiation of insulin therapy during hospitalisation and on an ambulatory basis. No differences were observed after one year. Now we evaluated the patients 3 years after the initiation of insulin therapy.

Results: Mean age of 122 patients 65.9 ± 8.8 years, years since diagnosis 12.3 ± 5.4 years. HbA1c values are expressed as relative values compared to mean normal value of non-diabetic persons (= 1).

Patients	**relative HbA1c**			**Body weight in kg**		
	Initially	1 year	3 years	initially	1 year	3 years
All pat. (122)	2.08	1.65*	1.61*	77.2	78.7*	80.6*
Ambul. (54)	2.08	1.63*	1.65*	78.5	79.3$^{\pi}$	79.7$^{\pi}$
Inpatients (68)	2.09	1.67*	1.58*	76.1	78.2*	81.3*

$^{\pi}$= n.s. = * = $p < 0.05$ against initial values

Conclusions: Three years after the ambulatory initiation of insulin therapy the results are still similar compared to matched patients who participated in the same programme on an inpatient basis. In contrast to the UKPDS, HbA1c did not increase but remained constant between one and three years after the initiation of insulin therapy. This may be explained by the structured teaching and treatment programme, resulting in a high compliance to self monitoring and self adaptation of the insulin dosage by the patients.

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THE IMPACT OF A CULTURALLY ADAPTED EDUCATIONAL PROGRAM ADDRESSED TO PAKISTANIAN IMMIGRANTS WITH TYPE 2 DIABETES.

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Aims: Due to a persistent problem of significantly worse glycaemic control in ethnic groups compared to the Danish population an educational initiative was carried out: 1) to assess the effect of a culturally adapted educational program addressed to Pakistanian immigrants with type 2 diabetes and 2) to inform the Pakistanian population in our area that they have a special health problem,as up to 20-25 per cent in their group living in western industrial countries have type 2 diabetes. **Materials and Methods**: Media were taken in use: local ethnic newspapers, radio and TV. Four information meetings were performed. The GP's of the area were informed about the possibility of transferring their Pakistanian patients to the culturally matched group education. Furthermore all immigrants from Pakistan who already frequented the Out Patients' Clinic were invited to participate in the group sessions. The sexes were separated : 15 men and 12 women with a mean duration of type 2 diabetes of 7.6 (SD 7.8) years were participating in groups of 4-8 members, 2 hours weekly for 5-6 weeks. During these sessions a culturally adapted educational concept was evolved : 1) a guide for diabetes educators containing usefull wordings, 2) a leaflet for the patient and the Pakistanian family and 3) high quality visuals assisting the learning. **Results:** contrary to former experience the attendance was now 95 % and 85 % for men and women respectively. Along with the group sessions the mean HbA1c (ref. 4.2-6.4%) for women was reduced from 9.7% (SD 1.3) to 9.0% (SD 1.3) (P=0,013) and for men from 10.7% (SD 2.7) to 9.5% (SD 2.2) (P=0,001). After a period of six to twelve months the HbA1c's were retested. Compared with the initial values the glycaemic control was now for women: 9.1 % (SD 1.5) (p=0,130)) and for men: 10.7 % (SD 3.0) (p=0,255),. **Conclusions:** this culturally adapted educational program is effective, but mainte-nance of knowledge is essential for keeping good glycaemic control.

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TEACHING AND TREATMENT PROGRAMMES IN TYPE 2 DIABETIC PATIENTS WITH IMPAIRED NEUROPSYCHOLOGICAL FUNCTION-AN INTERVENTION TRIAL

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Following the St. Vincent Declaration action programme all the patients with diabetes should take part in treatment and teaching programmes (TTP). In two pilot-studies with a total of 259 elderly type 2 diabetic patients we found that more than 50% of all the patients successively attended our clinic and participated in a TTP showed impaired neuropsychological function with partial learning disabilities. Hence, this seems to be the most important reason for patients' disability for diabetes self-management with resulting poor quality of diabetes care and high incidences of complications. From July to December 1998 in all the patients with type 2 diabetes (n=62, age 64,5±8,1, diabetes duration 12,7±9,8 years, HbA1c 9,8±1,6%, HPLC, Diamat®, normal range 4,4-5,9%) successively attended our clinic for the participation in a structured TTP, not only a neuropsychologal examination with the assessment of the patients' laerning ability was performed, but also diabetes knowledge, the ability for self-management and patients' quality of life (according to Bott et al.) were studied. In the mean, patients' neuropsychological score was 79,1±8,8 (range, 100,0-47,0). 15/62 patients had scores below the 2. percentile (<73,0; 68,6±6,3). In these patients partial learning disabilities were found. After participating in the TTP these patients had less diabetes knowledge (scores: 9,4±3,6 vs 11,5±3,1 in the other patients, p=0,032) and lower results in the questionnaires to assess the ability for diabetes self-management (16,6±5,3 vs 20,4±4,4 pts., p=0,008). There were no differences between the groups as regards age, diabetes duration, HbA1c or actual blood glucose. Performing multivariate analysis, there were strong associations between patients' age (c=-0,21, p=0,0043), the scores of the neuropsychological function (c=0,14, p=0,036) and patients' ability for self-management (R-square=0,19). Following these results before participation in a TTP it seems to be mandatory to assess patients' neuropsychological function and learning ability. Elderly patients with impaired neuropsychological function and partial learning disabilities should participate in modified TTP's. Such TTP's must consist in more exercise, more time for training of self-management and repeatment. Based on our findings in a prospective, randomized trial with 100 elderly patients and impaired neuropsychological function and partial learning disabilities the previous and a modified TTP will be compared. The first results will be presented.

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MANAGING TYPE 2 DIABETES BY GROUP EDUCATION: A 2-YEAR FOLLOW-UP.

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Aims: Most busy and/or understaffed clinics offer patients hurried consultations, which are unlikely to result in appropriate everyday health conducts. We verified if conventional doctor-patient consultations can be substituted by sessions of group therapeutic education (GTE), with the aims of: 1) prolonging the time spent by patients with the health care team while not increasing working hours, 2) eliciting favourable group dynamics, and 3) empowering patients to cope with everyday situations that pose risks for people with diabetes. **Patients and Methods**: Controlled randomized clinical trial, 2-year follow-up. Informed consent was obtained from 112 non insulin treated patients. Fifty-six were allocated to 6 education groups, for which a detailed programme, materials and plans had been prepared beforehand, while 56 controls continued formal consultations. Both GTE sessions and conventional visits were held 3-monthly, the former lasting about 1 hour and the latter about 15 minutes. **Results**: At baseline, GTE and control patients had similar HbA_{1c} (7.21±1.34 and 7.36±1.43, respectively), but BMI was higher in the former (29.8±4.3 vs. 28.0±4.3, p=0.03). After 2 years, patients on GTE, but not controls, had decreased their BMI (29.0±4.5, p=0.0014 vs. baseline) and their HbA_{1c} was lower than that of the controls (7.47±1.34 vs. 8.31±1.76, p=0.015), among whom it had worsened (p<0.0001). GTE patients had also increased HDL cholesterol (49.6±12.2 vs 52.9± 13.8, p<0.0005) and improved quality of life (initial and final DQOL questionnaire scores: 70.95±19.97 vs. 56.05±15.49, respectively, p<0.0001), knowledge of diabetes (GISED questionnaire, 14.86±7.87 vs. 25.20±5.76, p<0.0001), and induced more appropriate health conducts, as assessed by a purpose-built questionnaire (*Condotte di Riferimento*, 11.02±2.71 vs. 16.36±2.45, p<0.0001). Questionnaire scores did not change significantly among the controls. The time spent to see patients in GTE, check their blood test results and case notes before sessions, and examine those in need of individual attention was not greater than that spent to examine the control subjects. The patients found GTE sessions stimulating and the health personnel enjoyed a less repetitive approach to care. **Conclusions**: structured group education may be not simply a useful adjunct to traditional consultations but be effective on its own in reducing weight and maintaining good metabolic control in the medium term.

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A MULTIDISCIPLINARY INTENSIVE EDUCATION PROGRAM (M.I.E.P.): EFFECT ON METABOLIC CONTROL, SYMPTOMS AND QUALITY OF LIFE IN DIABETES (A PILOT STUDY).

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Summary:

Introduction: Conventional care of patients with DM exists of regular visits to an endocrinologist/internist, a diabetes nurse, a dietician and when necessary completed with a paramedical professionals. In most patients this conventional care system is sufficient. However, in a part of the population persistent worse metabolic control exists possibly caused by psychosocial problems.

Methods: We started a Multidisciplinary Intensive Education Program (10 days in 5 weeks, 2 refreshmentdays 6 and 12 weeks afterwards), designed to provide intensive care for this difficult group of patients, focussing on changing diabetes related behaviour and health locus of control, performed by a team of medical and paramedical diabetes workers. The targets of the program were to optimise metabolic control (HbA1c), to reduce the number of diabetes related symptoms (Diabetes Symptom Checklist; DSC type 2) and to obtain a higher quality of life (RAND-36). These parameters were obtained before starting of the program 12 weeks after finishing.

Results: Seventy-three patients were evaluated (male 34, female 39, mean age 48.9 years; SD 14.2). HbA1c improved in the whole group (8.13 % vs. 7.62 %; $p < 0.001$). The number of diabetes related complaints was low, nevertheless a significant improvement in fatigue and cognitive distress was found in patients < 40 years ($p < 0.001$) and in male ($p < 0.05$).With respect to quality of life, the patients experienced only few limitations in physical functioning at baseline. Patients < 40 years and patients with high education were limited in social and mental functioning, which improved after the program ($p < 0.01$ and $p < 0.05$). The health perception increased in the whole group ($p < 0.0001$), vitality increased in patients < 40 years or with high education (both $p < 0.05$). An increase in Internal Health Locus of Control in the whole group was found, especially in patients between 40-60 years ($p < 0.0001$) and in men ($p < 0.001$); a decrease in External Health Locus of Control was found in patients between 40-60 years ($p < 0.01$).

Conclusions: This explorative cohort study suggests that MIEP is effective concerning optimising metabolic control, reducing diabetes related symptoms and improving quality of life by influencing behaviour, even three months after finishing the program.

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ACUTE DIABETIC COMPLICATIONS IN PATIENTS RECEIVING EMERGENCY MEDICAL TREATMENT

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Aims: In order to improve the often inadequate quality of treatment given to diabetic patients in emergency situations and as a means of obtaining reliable information on the prevalence of such events, we established a standardised diagnostic and treatment procedure for an emergency catchment area in Germany covering 160,000 inhabitants. **Methods:** Emergency-response doctors and paramedics first received a one-off intensive course of instruction from a diabetologist on the diagnosis and treatment of severe hypoglycaemia and diabetic coma. Subsequent to that, during the period 1.1.1997 to 20.3.1999, a rapid, reflectometric blood glucose test from venous blood was carried out on all patients within the catchment area who received emergency medical treatment (exceptions were: patients who were already dead, those requiring reanimation, and young children). **Results:** Blood glucose was determined in 3599 patients (84%; age 56.7 ± 43 years; 54% male; glucose 7.15 ± 3.7 mmol/l) of the 4283 patients receiving emergency treatment. The prevalence of diabetic emergencies in the entirety of emergency-response callouts was 3%; 100 severe hypoglycaemias and 14 diabetic comas were recorded. Comparison with the 19588 emergency medical callouts in the years 1984 to 1996 in the same catchment area revealed a steady rise not only in the total number of emergency medical responses but also in the incidence of diabetic emergencies. The quality of treatment also improved. In severely hypoglycaemic patients the amount of 40 % glucose administered i.v. was raised (40 ± 20 ml versus 28 ± 20 ml; $p < 0.001$), those with sulphonylurea hypoglycaemias were infused with further glucose and clinically monitored. Following hypoglycaemic events in patients under CSII, the insulin pump was disconnected. In some cases, paramedics arriving first on the scene ahead of the doctor instituted effective measures by administering glucagon s.c. or glucose i.v. **Conclusion:** Training the emergency response team significantly improved the quality of treatment given in diabetic emergencies.

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Lipohypertrophy in insulin treated patients and some questions marks.
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Aim of the study was to identify some factors which may lead to lipohypertrophy in insulin treated patients. **Methods** : 55 patients, insulin treated and diagnosed as having lipohypertrophy were questioned and examined about it. They have been randomized of 130 insulin treated patients with lipohypertrophy. Lipohypertrophy was apreciated as palpable and visible subcutane-ous swelling larger than 1 x 1 cm in a single or multiple injections sites. **Results:** female-29.1%, male-70.9%, age: 26.72+- 12.86 years (range: 4...59 years), diabetes duration: 123.31 months (2...432 months). More than 90% of patients were normal and underweight persons. 7.27% patients presented HBA1c < 7.5%, 12.7% had HBA1c between 7.5 and 8%, and the rest had more than 8%. 74.5% of patients had no proper insulin injection technique. 40% of patients used more than 20 times a needle of pen or a syringe. 43.63% of patients and 41.81% received three, respectively four insulin injections per day. 80% of patients had no rotation of insulin injection sites. 89.09% experienced more than two sites of lipohypertrophy. 92.7% were diagnosed with hypertrophyc form. 91.3% of patients with multiple sites of lipohypertrophy received more than two standard educational programs as inpatients. **Conclusions**: Lypohypertrophy might appear even after two months of insulin therapy, but usually within 5..7 years. Lipohypertrophy seems to be related with improper injection technique and no rotation of insulin injection recommended sites. It seems to be no relationship between lipohypertrophy and the number of insulin injections within a day . HBA1c had a value greater than 8% for more than 80% of patients. It seems to ask for a further education and does not seem to be related with the presence of lipohypertrophy. A complementary specialised education process as well as individual instruction especially for children and underweight persons is needed in order to minimize this insulin therapy complication..

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SELF MONTORING BLOOD GLUCOSE AND METABOLIC CONTROL – AN UNCLEAR RELATIONSHIP
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Aims: Self-monitoring blood glucose (SMBG) is a cornerstone in diabetes treatment. A link between SMBG and level of metabolic control is, however, not clearly established. We therefore assessed the possible associations between patient characteristics, patterns and quality of SMBG and level of metabolic control measured with HbA_{1c}.
Materials and Methods: 422 patients with were included from a hospital outpatient clinic (n=159) and general practice (n=263). 177 had type 1 diabetes and 237 type 2, 8 had unknown type. Mean diabetes duration was 17 years for type 1 and 9 years for type 2. All patients performed two self-measurements of blood glucose (BG) with their own meters, responded to a questionnaire about SMBG and had blood samples drawn for HbA1c analysis. Analytic quality of BG measurements was assessed by comparison with a glucose dehydrogenase reference method. Effects of the variables on metabolic control was measured by chi-squared tests and logistic regression analysis adjusting for age and sex.
Results: 48% performed SMBG >3 times/week and 50% claimed to be self-educated in SMBG. 75% consider their meter to be reliable and 63% did not perform meter controls. Proportions of age, gender, diabetes type, SMBG frequency or educational mode were not significantly different from expected at defined HbA_{1c} -levels. Patients with a SMBG history of 6 years or less had a significantly lower HbA_{1c} ($p<0.05$). A SMBG precision level of 20% or better significantly increased ($p<0.005$) the chance of reaching HbA_{1c} <9%.
Conclusions: SMBG educational mode, SMBG frequency, type of diabetes or SMBG beliefs seem to have limited influence on HbA_{1c} -level. High SMBG analytic precision can possibly increase chances of a lower HbA1c. Educational efforts in SMBG are thus warranted, focusing not only on analytic performance but on actions to be taken by people with diabetes as well.

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PREVENTION OF SEVERE HYPOGLYCAEMIA IN TYPE 1 DIABETES CHILDREN. A PROSPECTIVE INTERVENTION STUDY 1995-98.
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Aims: Incidence of severe hypoglycaemia is high also with modern treatment, and may peak in spring season. May a prevention strategy using self-study material reduce severe hypoglycaemia, with unchanged or improved metabolic control?
Patients and Methods: The yearly 122-132 patients of an intensively treated geographic open population aged < 19 years, with age at onset 0.5-16.9 years (mean 7.5, SD 4.0) were asked to register every severe hypoglycaemia with unconsciousness (U) and deliver data at visits quarterly. *In early 1997*, two letters and two self-study brochures regarding diabetes treatment and prevention of severe hypoglycaemia were mailed to all. Yearly mean HbA1c levels and U incidences were calculated before-after, and incidences corrected for missing registration periods (2-8 % yearly).
Patient's attitudes to the intervention were measured by open questions and visual analogue scales in 1998.
Results (Mean±SD, *** $p<0.0001$):

Year	n	Age	Duration	HbA1c	U/pat	% with U
1995	122	12.5±4.4	4.9±3.7	7.1±1.1	0.17	12
1996	129	12.4±4.3	4.8±3.7	7.3±1.2	0.22	12
1997	132	11.9±4.3	4.6±3.8	7.0±1.1	0.15	10
1998	129	11.8±4.1	4.3±3.6	6.4±1.1***	0.14	7

Patient's attitudes to the intervention were predominantly positive. U events in spring/early summer period tended to decrease.
Conclusion: Mailed letters and self-study brochures regarding diabetes treatment and prevention of severe hypoglycaemia may be a useful complement to regular visits and contribute to improved metabolic control.

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CONTINUOUS CARE AND EDUCATION FOR TYPE 1 DIABETIC PATIENTS. ONE-TO-ONE CONSULTATIONS OR GROUP SESSIONS?

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Aims. The clinical effectiveness of a 3 years follow-up of interactive group sessions (IGS) was evaluated in 40 newly diagnosed insulin-dependent diabetic patients comparing to 40 controls followed in one-to-one standard routine consultations (SRC). Design: IGS were developed on quarterly scheduled group meetings (± 10 patients with relatives and Health Care Providers) to check metabolic parameters and to exchange perceptions, feelings and experiences on coping with diabetes by using problem solving techniques and a patient-centered approach. SRC consisted on quarterly routine consultations with the specialist where direct counseling were giving according to individual needs. Diabetes knowledge and feelings were measured by validated questionnaires and metabolic parameters were taken from the clinical record. Results: After 3 years, diabetes knowledge, skills and behaviours had significantly improved in both group but patients in IGS scored consistently higher ($p < 0,001$) than those attending SRC. Feelings on treatment responsibility, self confidence and autonomy were significant higher in IGS comparing to SRC (($p < 0,000$). HBA 1c mean levels have decreased from 12,4 to 6,9% in IGS vs 11,9 to 8,6% in SRC ($p < 0,002$). There also were less diabetic emergencies in the IGS. Conclusion: Continuous diabetes care by IGS was more efficient to develope knowledge and skills and to empower patients to cope with diabetes daily care improving the blood glucose levels and diminishing the need of emergency services.

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Psychosocial Aspects

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DIABETES COGNITIONS QUESTIONNAIRE (DCQ): DEVELOPMENT, PSYCHOMETRIC PROPERTIES AND CLINICAL APPLICATIONS.

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Aims: To develop and validate the Diabetes Cognitions Questionnaire (DCQ), a self-report measure designed to assess the cognitions associated with adjustment to diabetes. Certain cognitive content is associated with poorer psychosocial functioning in individuals with chronic medical illnesses, such as diabetes. Cognitive theorists argue that poor psychological adjustment is mediated by the interpretations and attributions that diabetes patients make about their condition. Evidence indicates that depressed and non-depressed diabetes patients can be distinguished by their cognitive responses. Accordingly, the DCQ was developed to facilitate both research and therapy activities that involve cognitive responses of these patients. Specifically, the DCQ may enhance our knowledge about the role of cognitive style in coping with diabetes, assist clinicians in the identification of maladaptive cognitive responses in diabetes patients, and serve as a useful measure of change in outcome research on the application of psychological approaches in the management of diabetes. **Method:** Items for the DCQ were generated on the basis of clinical experience obtained during interviews with patients with diabetes. The original DCQ (64 items) was administered to 76 individuals with non-insulin dependent diabetes mellitus (NIDDM), and was subsequently revised (30 items) and administered to a further 85 NIDDM patients. **Results:** The DCQ possesses high internal consistency (Cronbach's alpha = .88). Factor analysis yielded two factors: Negative and Positive Cognitions. Internal consistency for the two factors was found to be high (Negative = .93; Positive = .94). Item- total correlations for the two subscales exceeded .55. **Conclusions:** The DCQ has sound psychometric properties and is currently being used as an index of cognitive style in a cognitive-behavioural therapy study for patients with NIDDM. Research and clinical applications of the DCQ will be discussed in terms of integrating cognitive responses into more effective management of adjustment to diabetes.

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PSYCHOMETRIC PROPERTIES OF THE WORLD HEALTH ORGANISATION QUALITY OF LIFE QUESTIONNAIRE (WHOQOL) IN DIABETIC PATIENTS

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The aim of the study was to analyse psychometric properties of the WHOQOL-100, a multidimensionally conceptualized, generic, 100-item quality of life instrument in a pilot sample of 64 NIDDM patients (age 60.3+/-10.1 yrs; 54% women; disease duration 10 +/-5.2 yrs; education 10+/-5.2 yrs; 78% married). While covering the subjective perception and evaluation of overall QOL and general health, as well as 4 broad domains (psysical health, psychological state, social relationships, environment), the instrument may be useful in integrating the individual's QOL perception. The properties tested referred to reliability, validity in discriminating patients with different disease characteristics, responsiveness to change and convergent validity. **Methods:** Reliability was determined by Chronbach's alpha coefficients of internal consistency. Discriminant validity was determined by comparing the groups of patients with poor and satisfactory control, the first of them being switched to insulin therapy (intervention group-I vs control group-C, each consisting of 32 patients). Responsiveness to change was determined by comparing the two groups after a two-month follow-up period. Convergent validity was determined by comparing the WHOQOL data with data collected with the WBQ (C.Bradley). **Results:** The obtained alpha coefficients were 0.70-0.79 for the Social domain, 0.80-0.89 for the Psychological domain and 0.90-0.99 for the domains referring to Physical health and Environment. The groups characterized by different disease characteristics (I test vs C test) rated their QOL differently, primarily in the physical and psychological domain ($p<0.01$; $p<0.05$). After a two- month follow-up period the intervention group improved some quality of life determinants (I test vs I retest; $p<0.05$ for the Psychological domain), while no differences were found in the group which had not changed diabetes therapy (C test vs C retest; all $p>0.05$). The obtained correlations between the WHOQOL and the WBQ were statistically significant (general WB- WHOQOL: 0.78 (Physical domain); 0.80 (Psychological); 0.66 (Social relationships) and 0.78 (Environment). The correlations between the WHOQOL domains and the WBQ subscales were statistically significant and in expected directions. **Conclusion:** The WHOQOL-100 is a reliable and valid instrument for the QOL assessment in diabetic patients.

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IMPROVEMENT OF THE QUALITY OF LIFE AFTER AN EDUCATIONAL PROGRAM IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Evaluation studies of the impact on the quality of life (QOL) of diabetes educational programs (DEP) are scarce, especially at a primary care level. **Aim**: To study the impact of a DEP on the QOL in type 2 diabetic patients attended at a primary care unit (PCU). **Methods**: In 1998, 113 type 2 diabetic subjects were selected out of 1022 subjects with diabetes in a PCU in an urban area (28000 people). Exclusion criteria: Age > 75 yr, dementia, deafness, severe late diabetic complications. DEP was performed according to the DESG recommendations. Before and 3 months after performing the DEP, the following data were recorded: BMI, fasting plasma glucose, lipid profile, HbA1c, symptoms of anxiety and depression (HADS test), knowledge about diabetes (ECODI test) and QOL (SF-36 and DQOL tests). Data on sex, age, diabetes duration, treatment, monitoring, and late diabetic complications were also collected. **Results**: Five patients were excluded (irregular class attendance). 108 subjects were evaluated (47 women, 64.8±0.8 yr, 8.5±0.8 yr of diabetes, 29.2±0.7 kg/m^2, 16 with retinopathy, 38 with nephropathy and 13 wih ischaemic heart disease, 71 on glucose self-monitoring, 17 on insulin treatment). After the PED, of the 8 SF-36 scales, the scales of general mental health (66.7±2.4 vs 71.9±2.3, p=0.05) and general health perceptions (54.6±2.1 vs 58.8±2.2, p=0.02) improved and the remaining scales showed no change. Of the 4 DQOL scales only the scale of satisfaction improved (33.5±1.1 vs 29.9±0.9), whereas the others did not change. An improvement in HbA1c (6.7±0.1 vs 6.2±0.1%, p=0.04) and in diabetes knowledge (15.3±0.5 vs 18.9±0.4, p=0.00) but not in weight loss was observed. There was no correlation between improvement in QOL scales and improvement in HbA1c and diabetes knowledge. Improvement of QOL was not related to late complications, monitoring or treatment. **Conclusions:** DEP improved several scales of the QOL independently of its beneficial effect on metabolic control and diabetes knowledge.

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COMPARING CRITERION REFERENCED QUALITY OF LIFE SCORES WITH SOCIODEMOGRAPHIC AND DISEASE RELATED VARIABLES IN TYPE 2 DIABETES

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Aims: To determine the criterion validity of the SF-36 health survey in type 2 diabetes, to compare quality of life (QOL) scores of groups identified by criterion referenced scoring with the SF-36 and examine possible relationships between these scores with certain sociodemographic and disease related variables. **Materials and Methods:** 187 type 2 diabetic (hospitalized and outpatients) were interviewed using the SF-36 health survey to assess health status and related QOL. Second questionnaire was applied to determine general sociodemographic and disease related independent variables.Information about metabolic and clinical variables were obtained from the medical records. Criterion validity was assessed by dividing patients into two groups (group 1 good health, group 2 poor health) according to their answers to the first item of the SF-36 questionnaire, asking respondents to evaluate their overall health, and comparing the scores of these two groups for the remaining seven SF-36 multi-item scales determining functional status and and well-being. **Results:** worsening self rated health was statistically significantly associated with decreasing SF-36 scores, indicating greater health problems.Bad health perception was also associated with a higher incidence of chronic diabetic complications and sedantery lifestyle, whereas the metabolic profile of both groups was rather similar. Indicators of decreasing QOL perception for group 1 evaluation were more than one comorbid condition besides diabetes and the presence of diabetic relatives in the family. Poor educational level and type of therapy (combined) were adversely affecting QOL perception of patients who rated their health as poor.For both groups decreasing SF-36 scores were mainly associated with the presence of chronic complications (nephropathy with hypertension). **Conclusions:** The SF-36 health survey, which is a generic QOL questionnaire, combines both: a wide scope and high psychometric qualities such as internal criterion validity and sensitivity to change, thus becoming as eligeable in diabetic health related QOL outcome research as disease specific instruments.

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QUALITY OF LIFE IN DIABETIC NEPHROPATHY: COMPARISON OF GENERIC VS CONDITION-SPECIFIC MEASURES.

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In the few previous studies assessing quality of life (QOL) in diabetic nephropathy, generic measures were invariably used, but there is interest in determining whether condition-specific measures are more able to detect subtle variations in QOL. Aims: to compare the performance of a generic scale (SF 36) with a condition-specific scale of known validity (Renal Quality of Life Profile:RQLP) and to assess QOL in patients with differing degrees of renal dysfunction. Methods: Four age-matched groups were studied: group A (n= 19): diabetic patients on peritoneal dialysis (CAPD); group B (n=26): non-diabetic CAPD patients; group C (n=20): diabetic patients with renal transplants, and group D (n= 20): diabetic patients with impaired renal function. No differences in other complications or co-morbidities were observed across the diabetic groups. Results: the worst QOL was reported in CAPD patients on some SF 36 and most RQLP domains, compared to groups C and D: e.g., SF 36, social functioning: $p<0.05$; RQLP, eating/fluid intake: $p<0.001$. Diabetic patients on CAPD had worse QOL compared with non-diabetic CAPD patients, e.g., RQLP, leisure domain: $p<0.05$. QOL of transplant patients was as good as that in group D patients. The subscales of the two measures variably correlated with each other (range r=-0.04 to 0.67), indicating only a modest degree of overlap, and RQPL discriminated better between the groups. Conclusions: 1) this first study comparing measures in renal patients confirms that each examines QOL from differing but complimentary perspectives, supporting their use in combination, and 2) diabetes imposes an extra burden on QOL in patients with renal disease.

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DO PEOPLE WITH DIABETES DESIRE A ROLE IN DIABETES TREATMENT DECISIONS?

D.A. Gormley[1], A.K. Baksi[2] and the WHO/IDF Patient Empowerment Workshop, [1]Collegeville, PA, USA; [2]Isle of Wight, UK

Aims: The St. Vincent Declaration stated that the goals of improving the health and preventing complications in people with diabetes would only be achieved with the active participation of such individuals through patient empowerment. In an effort to measure current attitudes of diabetes patients towards patient empowerment, a survey was conducted in the UK. **Material and Methods:** A mail survey was conducted in the UK in July 1998 with a national sample of 453 diabetic patients. Respondents were randomly selected from national household survey lists and telephoned to determine their willingness to participate in the study. They were then sent a self-completion questionnaire to fill out and send back. **Results:** Approximately 90% of diabetes patients treated with insulin only and 80% of patients treated with oral agents for diabetes felt they should be told about diabetes treatment options by their physicians and should be involved in treatment decisions. The majority of patients felt confident they were capable of making some choices themselves regarding their treatment. Insulin users (91%) were more likely than oral medication users (78%) to feel confident about making some choices themselves. However, less than half of insulin-only patients and less than 15% of orally treated patients felt they knew more about how to control diabetes than their professional caregivers. **Conclusions:** Most people with diabetes want their health-care professionals, especially physicians, to discuss and involve them in diabetes treatment care decisions. Patients still look to their health care professionals as the ultimate experts in this area. The WHO/IDF Patient Empowerment Workshop believes the quality of diabetes care will improve if more patients and physicians openly discuss diabetes treatment options together and patients are allowed a role in treatment decisions.

	% agreeing "somewhat" or "strongly" with statement	
	Insulin Users (n=169)	Oral Med Users (n=284)
I should be told about treatment options	87	80
I should be involved in treatment options	91	78
I feel confident to make some choices myself	90	71
I feel I know more about how to control diabetes than most professionals	46	14

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HOW CONCERNED ARE PEOPLE ABOUT THEIR DIABETES?

A.K. Baksi[1], D.A. Gormley[2] and the WHO/IDF Patient Empowerment Workshop, [1]Isle of Wight, UK; [2]Collegeville, PA, USA

Aims: The St. Vincent Declaration stated that the goals of improving the health and preventing complications in people with diabetes would only be achieved with the active participation of such individuals through patient empowerment. In an effort to measure current attitudes of diabetes patients towards patient empowerment, a survey was conducted in the UK. The survey included questions regarding feelings about diabetes. **Material and Methods:** A mail survey was conducted in the UK in July 1998 with a national sample of 453 diabetic patients. Respondents were randomly selected from national household survey lists and telephoned to determine their willingness to participate in the study. They were then sent a self-completion questionnaire to fill out and send back. **Results:** Three-quarters of these randomly selected patients somewhat or strongly agreed with the statements "I feel in charge of my diabetes" and "My diabetes is well-controlled". Patients treated with insulin or oral agents were equally likely to agree with these statements. Insulin users were more likely to indicate worrying about future health problems than oral medication users (69% vs 51%). About one half of patients agreed that diabetes has required a lot of adjusting of their lifestyle. Oral medication users were somewhat less likely to state that their lifestyle has required a lot of adjusting (50%) compared to insulin users (55%). **Conclusions:** Many diabetes patients appear to believe that their diabetes is well under control. Future health problems are not a significant concern for 3 of 10 insulin users and 5 of 10 oral medication users. Only about half of diabetes patients feel that they have made major changes to their lifestyle as a result of their diabetes. The WHO/IDF Patient Empowerment Workshop believes that outcomes in diabetes can be significantly influenced by patients' behavior. People with diabetes may find increased motivation to change their lifestyle through empowerment.

	% agreeing "somewhat" or "strongly" with statement	
	Insulin Users (n=169)	Oral Med Users (n=284)
I feel in control of my diabetes	77	78
My diabetes is well controlled	77	75
I worry about future health problems	69	51
My lifestyle has required a lot of adjusting	55	50

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EATING DISORDERS IN YOUNG FEMALES WITH IDDM

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Aims: To identify the eating habits and attitudes in young adult women with IDDM compared with non-diabetic control subjects. **Materials and Methods:** A total of 86 IDDM female patients and 135 female control subjects were examined by the Eating Attitude Test (20-item EAT, Garner, 1991) and the Eating Disorder Inventory (EDI Garner, Olmsted, Poilivy, 1983). The mean age of the group of diabetic patients was 25.5 ± 5.6 yrs; mean HbA1c 8.9% ± 2.4; mean duration of diabetes 11.6 ± 6.2 yrs, mean BMI (kg/m^2) 23.6 ± 3 (versus 22.1 ± 3.7 in healthy controls). The two groups did not differ in age, height, marital status, and education. **Results:** In the Eating Attitude Test, diabetic patients showed significantly more often ($p < 0.001$) disturbed eating attitudes; in addition, they exhibited a more marked tendency toward a dietary behaviour ($p < 0.0001$) and bulimia ($p < 0.05$) than healthy females. In the test, 16% of diabetic patients had a higher than the critical score which is considered pathognomic for eating disorders, another 6% of females showed values very close to the critical score (overall, 22% of diabetic patients). A positive correlation ($r = 0.36$, $p < 0.01$) between HbA1c and the EAT score indicates a relationship between the degree of diabetes control and disturbed eating attitudes. Female diabetic patients report a statistically significantly more frequent tendency to be deceptive about their diet ($p < 0.001$) and less intensive hunger. In the Eating Disorder Inventory, female diabetic patients score higher in Drive for Thinness ($p < 0.001$), Interpersonal Distrust ($p < 0.05$), and Ineffectiveness. **Conclusions:** The results of the study suggest diabetes is associated, in a high proportion of female patients, with disturbed eating, eating attitude and habits, which may lead to subclinical and clinical eating disorders.

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Title: PSYCHOSOCIAL PROBLEMS IN ADOLESCENT DIABETIC PATIENTS.
Authors: MC Gåfvels, BSW, PhD, F Lithner MD, PhD. University of Umeå and Karolinska Hospital, Sweden.
Aim: To investigate psychosocial/social problems and needs of psychosocial intervention in adolescent diabetic patients (18-22 years old) referred from a children's department to a diabetes unit for adults during a five year period.
Methods: A questionnaire exploring social situation, experience of diabetes, quality of life and self-esteem was given to all patients in the study group at their first visit to the diabetes unit for adults. They also met the diabetes team members, the medical social worker included. By means of a semi-structured interview, psychosocial problems were detected and needs of intervention were decided. For patients with problems that had a continuous contact with the social worker, the psychosocial treatment process was followed by means of a special form. The other patients were followed using data registered by other members of the team in conjunction with their ordinary diabetes check-ups.
Results: Fifty patients (52 % men) participated in the study. 38 % had a diabetic duration of >10 years. Twenty-four patients (46 % men) were considered to have psychosocial problems. Types of problems were eating-disorders, denial, fear of injections, family problems, work-related problems, financial problems, alcohol abuse and criminality. A few also had serious mental problems. Compared to the other patients, patients with psychosocial problems (PPP) more often came from a dysfunctional family. Fewer of them had a boy- or girlfriend (25 vs 41 %). They worried more about the disease and their future (58 vs 33 %). They also more often felt "different" than other people (50 vs 38 %) and tried to hide their diabetes.
Conclusion: In this study almost half of the young patients had problems that required different kinds of psychosocial interventions. For a favourable prognosis it is important that such problems are detected and treated as early in the course of diabetes as possible. Findings of this study indicate that a social worker and/ or a psychologist should be natural members of every diabetes team, especially those treating young people.

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SOCIAL STATUS AND PROGNOSIS OF PEOPLE WITH TYPE 1 DIABETES ON INTENSIFIED INSULIN THERAPY
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Aims: To assess standardized mortality ratios (SMR) and predictors of mortality and severe diabetic complications in type 1 diabetic patients on intensified insulin therapy in relation to social status. Methods: All 3674 patients (insulin treatment before age 31) who had participated in a 5-day inpatient treatment and teaching programme for intensification of insulin therapy between 9/1978 and 12/1994 were followed for 10 ± 3 (mean ± SD) years; 50% were women, age at baseline 27 ± 11 yrs, diabetes duration 11 ± 9 yrs. Patients were divided into 3 groups according to social status, i.e. an additive variable of the highest educational level achieved and the present or last employment level. Results: Vital status and data on blindness, renal replacement therapy and amputation were available for 3570 (97%) patients; 251 (6,8%) patients had died. The following SMR were calculated by using the respective geographic area (North Rhine Westphalia) as reference population: Men: Social status I (highest), 2.15 (95% CI 1.53-2.94); social status II, 5.51 (3.86-7.63); social status III (lowest), 6.04 (4.57-7.83); Women: Social status I, 4.11 (2.71-5.98); II, 8.52 (5.13-13.31), III, 6.19 (4.08-9.01). Using the Cox proportional hazards model, the following risk factors of mortality as assessed at baseline were identified: nephropathy (at least macroproteinuria), smoking, diabetes duration, cholesterol, social status, age, sex, and systolic blood pressure. In addition, the following risk factors of the combined enpoint - blindness or renal replacement therapy or amputation – were identified: nephropathy, foot complications, HbA1c, smoking, cholesterol, systolic blood pressure, advanced retinopathy, antihypertensive drug therapy, and social status. Conclusions: Low social status is a risk factor of both mortality and severe diabetic complications among persons with type 1 diabetes on intensified insulin therapy.

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Gestational Diabetes

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SHORT STATURE AND GESTATIONAL DIABETES: VARIABILITY BY LEVEL OF ADIPOSITY.
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Aims: To examine the association between maternal stature and gestational diabetes mellitus (GDM). **Materials and Methods:** . We performed a 2h 75g oral glucose tolerance test, defining GDM by World Health Organization criteria in 4997 consecutive Brazilian women ≥20 years old, pregnant for ~21-28 weeks, without history of diabetes outside pregnancy, attending general prenatal care units in six state capitals in Brazil from 1991 to 1995. **Results:** Those in the shortest quartile of height (≤151cm) had a 60% increase in the odds of having GDM, independently of prenatal clinic, age, global obesity, family history of diabetes, skin color, referral pattern, waist circumference, gravidity, previous gestational diabetes, education, ambient temperature, and gestational age when compared to the tallest quartile (p=0.005). For those with above-median skinfold thicknesses this association was highly significant (OR=1.74, P=0.006), but not for those with below median thicknesses (OR=1.22, p=0.51). We found a similar association for short stature with high 2h glycemia (≥7.8 mmol/l; OR=1.61, p=0.005), but not with high fasting glycemia (≥5.5 mmol/l; OR=0.97, p=0.90). **Conclusions:** Maternal short stature is independently associated with gestational diabetes. The association occurs for post-load glycemia but not for fasting glycemia,and for adipose women, but minimally for lean women.

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COMPARISON OF ANTEPARTUM AND EARLY POSTPARTUM CHARACTERISTICS AS PREDICTORS OF TYPE 2 DIABETES FOLLOWING GESTATIONAL DIABETES.
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Aims: To compare antepartum and early postpartum metabolic characteristics as potential predictors of type 2 diabetes in women with gestational diabetes mellitus (GDM).
Methods: We conducted glucose clamps, and intravenous (IVGTT) and oral (OGTT) glucose tolerance tests during the third trimester and OGTTs within six months postpartum in a cohort of Latino women with GDM. Cox regression analysis was used to identify independent predictors of diabetes during follow-up in 94 women without diabetes <6 months postpartum whose diabetes status was ascertained 8-52 months (median 39 months) later.
Results: The women developed diabetes at an average rate of 9.2% per year. When antepartum variables were considered alone, independent predictors of diabetes were: OGTT 1-hour glucose (higher=increased risk, p=0.0001) and OGTT early insulin response (lower=increased risk, p=0.015). When postpartum variables were considered alone, independent predictors of diabetes were: OGTT glucose area (higher=increased risk, p=0.002), OGTT early insulin response (lower=increased risk, p=0.01) and body mass index (BMI, higher=increased risk, p=0.014). When antepartum and postpartum variables were considered together, three independent predictors were: antepartum OGTT 1-hour glucose (higher = increased risk, p<0.0001), postpartum OGTT early insulin response (lower=increased risk, p=0.001), and postpartum fasting insulin (higher=increased risk, p=0.04).
Conclusion: Early postpartum measures of poor B-cell function (OGTT insulin response) and insulin resistance (high fasting insulin) were major predictors of type 2 diabetes after GDM during a median follow-up of 39 months. The findings highlight the importance of poor B-cell compensation for chronic insulin resistance as an early defect in the pathogenesis of type 2 diabetes after GDM.

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HEALTH REALTED QUALITY OF LIFE IS ASSOCIATED WITH INSULIN LISPRO USE IN GESTATIONAL DIABETES MELLITUS (GDM)

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Aims: Insulin therapy is required when normoglycemia is not achieved by diet alone in GDM. This study assessed the behavioral response to insulin therapy using the Health Related Quality of Life questionnaire including: 1. meal time flexibility, 2. activity/convenience, 3. perceived glucose control, 4. insulin injection lag time preference, 5. compliance with treatment and 6. patient satisfaction.

Methods: Nineteen women with GDM were randomized to lispro therapy, and 22 were randomized to human regular insulin. Glycemic control was evaluated by HbA_{1c}, home blood glucose measurements, and a prevalence of blood glucose <55 mg/dl and ≥120 mg/dl. The Quality of Life questionnaire was administered at the end of the study. We also assessed carbohydrate intake before and during insulin therapy. All patients were counselled to have 40% carbohydrates in their meal plans.

Results: Insulin lispro significantly improved HbA_{1c} and reduced hypo and hyperglycemic episodes without increased immunogenecity. Patients in the lispro group had significantly higher flexibility regarding the meal time and planning of daily activities (p=0.005, for each). Patients in both groups felt they had excellent glucose control with the insulin therapy. Only 11 (50%) women with GDM on regular human insulin were compliant regarding prescribed timing of insulin injections, but 14 (63.6%) would have preferred to have different timing. In contrast, 18 (94.5%) patients on insulin lispro were compliant (p<0.001). The preferred time for insulin injection for both groups was less than 10 min before meals. All woman were satisfied with their health education. Patients on regular insulin maintained their carbohydrate intake at the constant level (39.4±2.5% before, and 40.1±3.1% after insulinisation), whereas patients in the lispro group increased their carbohydrate intake (from 39.1±2.7% to 47.4±2.2%).

Conclusion: In conclusion, among women with GDM, insulin lispro not only improved glucose control, but also significantly increased compliance, probably due to the shortened lag time between injection and meal. Also, meal flexibility and activity of daily life were all improved with lispro.

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RISK FACTORS FOR INSULIN TREATMENT IN GESTATIONAL DIABETES MELLITUS.

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Aims: To detect risk factors for insulin treatment in women with gestational diabetes mellitus (GDM).

Patients and Methods: 197 pregnant women with GDM diagnosed according to the NDDG criteria were studied. Diet treatment (DT) was started in all patients and insulin treatment (IT) was added if preprandial capillary glucose levels were above 100 mg/dL and/or one hour postprandial glucose levels were above 140 mg/dL. Differences between DT and IT groups were assessed (x^2 test or t-test) and a logistic regression analysis was performed to investigate risk factors for insulin therapy.

Results: GDM in the IT group (n=34) was diagnosed earlier (27 ± 5 vs 29 ± 4 weeks of gestation, p<0.05) than in the DT group. IT patients were more obese (27.1 ± 5.0 vs 24.7 ± 4.7 kg/m^2, p<0.05), had higher basal and 1-h glucose values on the OGTT (94 ± 15 vs 84 ± 11 mg/dL and 221 ± 27 vs 206 ± 25 mg/dL, p<0.05) and higher frequency of first-degree relatives with diabetes than DT patients (47 % vs 26%, p<0.05). There were no differences in age, previous spontaneous abortions, parity, 2-h or 3-h OGTT plasma glucose levels. Continuous variables were then dicotomized, being cut-points for plasma glucose levels on the OGTT the mean + 1 SD of the values obtained in the DT group (95-231-209-176 mg/dL). Variables independently associated with a higher risk for IT were pregestational obesity (BMI ≥ 27 kg/m^2;RR2.38, 95% C.I.1.03-5.46), higher fasting and 1-h glucose values (RR 2.52, 95% C.I. 1.02-6.22; RR 2.70, 95% C.I. 1.08-6.75), and a positive family history of diabetes in first degree relatives (RR2.16, 95%C.I.0.95-4.3).

Conclusions: Obese pregnant women with higher fasting and 1-h blood glucose levels and with a positive family history of diabetes are at increased risk for requiring insulin therapy. These patients should be followed tightly to initiate insulin treatment as soon as it is needed.

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PREDICTIVE FACTORS FOR INSULIN TREATMENT IN WOMEN WITH GESTATIONAL DIABETES MELLITUS

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Aims: to compare clinical and metabolic parameters in two groups of GDM women: treated with diet alone (group 1) or with diet and insulin (group 2) and to determine predictive factors for the initiation of insulin treatment. Patients and methods: the data of 122 GDM women were retrospectively analyzed. The women underwent a diet containing 25 Kcal/Kg/24h and insulin was started when fasting glicemia and 2h postprandial were respectively > 105 or > 120mg/dl. 84 women were treated with diet alone and 38 were treated with diet and insulin. Results: the characteristics compared in group 1 and group 2 were respectively: age (yr.) 30.02±5.65 Vs 31.97±5.73 p=0.04, prepregnancy BMI (Kg/m2) 24.93±5.12 Vs 28.03±5.24 p<0.0001, fasting glicemia (mg/dl) 77.92±14.88 Vs 99.08±41.98 p<0.0001, glicemia at the screening test (mg/dl) 167.62±29.83 Vs 182.64±34.95 p<0.0001, 3h-100g OGTT at 0' 83.02±22.91 Vs 100.05±35.15 p<0.0001, at 1h 207.30±31.4 Vs 221.23±34.7 p<0.0001, gestational age at GDM diagnosis (wk.) 29.83±8.33 Vs 24.71±7.75 p<0.0001, HbA1c (%) 4.93±0.72 Vs 5.58±1 p<0.0001, neonatal birth weight (Kg) 3.210±0.55 Vs 3.338±0.48 p=0.05. Total cholesterol, triglicerydes and weight gain during pregnancy were similar in both groups. Logistic regression analysis showed that gestational age at GDM diagnosis (OR 0.88 [95% CI 0.80-0.97], p=0.015) and 0' (3h-100g OGTT) glicemia (OR 1.03 [95% CI 1.0-1.06], p=0.028) were the independent risk factors for the initiation of insulin treatment. Conclusion: the GDM women who started insulin therapy were older, had a higher prepregnangy BMI, fasting glicemia, glicemia at the screening test, at 0', at 1h-OGTT and HbA1c, when compared with the GDM women treated with diet alone. Gestational age at GDM diagnosis and glicemia at 0'-OGTT were the predictive factors for insulin treatment in gestational diabetes.

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MATERNAL AND FETAL OUTCOME IN GESTATIONAL DIABETES MELLITUS

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Aims: To analyze the clinical findings, the obstetric results and the post-partum metabolic evaluation in a group of women with Gestational Diabetes Mellitus(GDM).

Material and methods: We studied 354 women in which we evaluated age, parity, family history (FH) of Diabetes Mellitus (DM) and Hypertension Arterial (HTA), menstrual function, body mass index (BMI), initial HbA1c and in the last trimester of gestation, pattern of delivery, fetal results, and post-partum metabolic evaluation by TTOG.

Results: The mean age was 32,3±4,87 years; BMI of 26,7±5,23 k/m2. Diagnosis of GDM was stablised at the 25± 8th week of pregnancy and the initial value of HbA1c was of 4.99±0,6 %. In 244 patients (68,9%) there was a FH of DM type 2 and in 26 (7,3 %) of DM type 1. The FH detected antecedents of HTA and GDM in 55 (15,5%) and 34 (9,6%) patients respectively. There were 106 primigravidas (29,9%) and 248 multigravidas (70,1%) of which 94 (37,9%) had one or more previous abortions and 46 (18,5 %) had delivered previously one or more infants of more than 4000 gms birth weight. Previous GDM was found in 57 (22,9%). Menstrual hystory was anormal (oligomenorrea - secondary amenorrea) in 84 patients (23,7 %). HbA1c was 4,8±0,63 and 4,0±1,6 in the 8th and 9th month respectively. Obstetric results were 5 first trimeter spontaneous abortions, 12 preterm deliberies, an 337 term births. In 196 (55,4%)cases, the iniciation of labor was spontaneous, while in 102 patients (28,8%) it was induced. Cesarean section was performerd in 52 (14,6%). The incidence of macrosomic infants was 6,7 % (24 cases). We were able to perform metabolic assessment in 198 (55,9%) patients six months after delibery and we have diagnosed 9 cases of DM (4,54%) , and 48 cases of impared glucose tolerance (IGT) (24,2 %).

Conclusions: With this results, we have arrived at the following conclusions: 1)Presence of diabetes is very frequent in the family history of GDM. 2)A high incidence of multiparity between these patients. 3) Oligomenorrea –secondary amenorrea is frequent in patients with GDM. 4) The high prevalence of IGT detected in the post-partum control makes necessary to stablish a program for assessment of this entity.

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OUTCOME OF DIABETIC PREGNANCY IN RELATION TO ANTENATAL GLUCOSE TOLERANCE TEST

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Aim: To study the relation of antenatal fasting and 2-hour post glucose (75 gm), plasma glucose levels on diabetic pregnancy. **Patients and Methods:** Patients (n=228) attending the diabetic pregnancy clinic in three centres (Solihull, Birmingham and Manchester) were retrospectively entered into the study and data obtained from case notes. Gestational diabetes/impaired glucose tolerance was defined according to WHO criteria. All patients were seen regularly at the diabetic pregnancy clinic and had daily (x4) home blood glucose monitoring (BM). Treatment (diet or diet and insulin) was advised according to BM and glycosylated haemoglobin (HbA1c). **Results:** 50 of the 228 babies were macrosomic (>4kg). Fasting GTT and third trimester HbA1c was higher in the macrosomic group (5.9 ± 1.9 vs 5.3 ± 1.3 mmol/l; $p<0.05$; 5.6 ± 1.1 vs $5.1 \pm 0.9\%$, $p<0.005$); but not with 2-hour glucose or 2nd trimester HbA1c. Patients who had higher fasting and 2-hr GTT were more likely to be treated with insulin ($p<0.001$). Spearman's Rank correlation revealed significant correlation between birth weight and HbA1c in 2nd or 3rd trimester ($p<0.01$) and fasting GTT ($p<0.05$); birth weight did not correlate with 2-hr GTT. Fasting GTT was able to predict overt diabetes (2-hr GTT >11.1mmol/l) (area under ROC curve 0.741 ± 0.08) but not IGT (2-hr GTT≥7.8 and <11.1). **Conclusion:** Although fasting blood glucose can identify patients with diabetes in pregnancy it cannot be used to determine all patients requiring intensive monitoring. Therefore we conclude that a formal GTT is still indicated in the diagnosis of gestational diabetes.

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EVALUATION OF RISK FACTORS FOR THE DEVELOPMENT OF GESTATIONAL DIABETES MELLITUS (GDM) IN GREECE.

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Aims. Undiagnosed GDM is associated with increased risk of perinatal morbitity. Consequently, the early identification of women at risk is important. The aim of the present study was: a. to evaluate the recognised risk factors for GDM independently in a Greek population and b. to define the prevalence of GDM in a "low risk" subgroup. Materials and methods: 3513 Greek pregnant women without known DM underwent a 100g OGTT in the third trimester, between 1990-1998. For GDM diagnosis the Carpenter-Coustan criteria were used. Age, pre-pregnancy BMI, family history of DM, smoking habits before and during pregnancy and the number of previous deliveries were recorded. Adjusted for the above mentioned variables relative risks (aRR) and 95% C.I. were calculated by logistic regression. Results. Multivariate analysis showed that maternal age >25 years significantly increased the risk for GDM (p for trend <0.001). For age 26-30yrs: aRR, (95% C.I.) 2.26, (1.54-3.36), age 31-35yrs: 2.57, (1.53-4.32), age 36-40yrs: 4.17, (2.07-8.43), age>40yrs: 5.15, (2.03-13.07). Higher BMI also increased the risk for GDM (p for trend <0.001). BMI 20.1-22: aRR=0.96 (0.63-1.46), BMI 22.1-25: 1.27 (0.85-1.88), BMI 25.1-30: 1.55 (1.04-2.32), BMI 30.1-35: 2.99 (1.94-4.6), BMI >35 kg/m^2: 3.7 (2.3-6.1). Positive family history for DM significantly increased the risk to develop GDM: paternal history: aRR=1.33 (1.03-1.71), maternal history: 2.38 (1.88-3.02), history on both sides: 2.43 (1.37-4.33). Multiparity became a significant risk factor only when there were ≥4 previous deliveries: 2.19 (1.18-4.06). Smoking was not independently associated with an increased risk to develop GDM. Finally, 150/3513 women belonged to the "low risk" group (age <25, BMI <25, no family history for DM, non-smokers, first pregnancy). 12/150 (i.e.8%) were defined as GDM. Conclusions: Advanced age (>25 yrs) and higher BMI (>25) progressively increase the risk for GDM. Maternal family history for diabetes mellitus is a significant independent risk factor for GDM. Universal screening for GDM seems worthwhile including the "low risk" group, at least in the Greek population.

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GESTATIONAL DIABETES MELLITUS: IS SELECTIVE SCREENING WORTHWHILE?

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Fourth Workshop-Conference on Gestational Diabetes Mellitus (GDM) Recommendations introduce the concept of selective screening: it would not be required in women fulfilling all the following: ethnic group with a low prevalence of GDM, no diabetes in first-degree relatives, less than 25 years, normal weight before pregnancy and no history of either abnormal glucose metabolism or poor obstetric outcome. **Aims:** to assess if this policy is worthwhile in our center, analysing: 1) How would this strategy influence the detection of GDM and 2) What are the characteristics of women who would not be diagnosed. **Materials and Methods:** The first issue was studied in the 917 pregnant women delivering in the Center in 1992 (universal GDM screening, NDDG criteria). For the second issue we studied the whole cohort of 1635 women with GDM (delivery 1986-98) and compared the outcome between those where the diagnosis would be skipped (N = 21) or retained (N = 1614). **Results:** 1) In 1992, 7.0% women would not have undergone GDM screening, had it been selective. The main reason to not skip screening was age, as only 12.6% of the population was younger than 25. One hundred and ten women (12.0%) were diagnosed of GDM, two of whom would have been undiagnosed. 2) In women with GDM, the characteristics and outcome in women with potentially skipped or retained diagnosis are as follows: insulin Tx 38.1% vs 52.9%, hypertension 0% vs 6.5%, preterm delivery 4.8% vs 7.0%, caesarean section 4.8% vs 20.5%, 1 min Apgar <7 0% vs 6.5%, macrosomia 0% vs 4.4%, large for gestational age infants 0% vs 8.4%, small for gestational age infants 19%.0 vs 7.3%, neonatal hypoglycemia 5% vs 6.0%, hypocalcemia 0% vs 1.4%, hyperbilirrubinemia requiring Tx 0% vs 3.9%, respiratory distress 0% vs 2.9%, perinatal mortality 0% vs 0.5%, overall adverse outcome 31.3% vs 45.7% differences not reaching statistical significance in any case. **Conclusions:** We conclude that selective screening is reliable in identificating women at low risk of GDM but implies a more complex screening policy that would avoid screening in a negligible subset of the pregnant population.

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WHICH FASTING GLUCOSE CUT POINT SHOULD BE USED WHEN DIAGNOSING GESTATIONAL DIABETES ?

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A 2h glycemia of 7.8 mmol/l during a 75g OGTT has been recommended by World Health Organization committees for the diagnosis of gestational diabetes (GDM), although outside pregancy this value defines a milder than diabetes condition. However, for fasting glycemia, the values used outside pregancy – 7.8 mmol/l or, more recently, 7.0 mmol/l – have been recomended for GDM diagnosis. **Aims:** To evaluate fasting glucose cut points against 2h glucose values obtained during a standard OGTT in pregnancy. **Materials and Methods:** We enrolled 5564 consecutive Brazilian women 20 years or older without diagnosis of diabetes mellitus outside of pregnancy between their 21st and 28th gestational weeks, performing a 75g OGTT on 4997. **Results:**

2h Glucose (mmol/l)	Percentile (%)	Fasting Glucose (mmol/l)
7.8	93.3	5.5
11.1	99.7	6.7
11.7	99.8	7.0
12.3	99.9	7.8

A fasting value of about 5.5 mmol/l corresponds, percentilewise, to a 2h value of 7.8 mmol/l. **Conclusions:** WHO fasting cut points for diagnosis of GDM of 7.8mmol/l, and even of 7.0mmol/l, are too high to be useful during pregnancy. Although 2h and fasting values still require validation against clinical outcomes, a lower fasting value may prove more appropriate.

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THYROID AUTOIMMUNITY IN CHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES

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Introduction. The association of type 1 diabetes mellitus (IDDM) with autoimmune thyroid diseases (ATD) has been well described in many works, but there is little about the clinical course of patients with biochemical signs of thyroid autoimmunity, especially in pediatric age. **Aim of the study.** Evaluating thyroid autoimmunity and the prevalence of ATD in IDDM pediatric patients. **Patients and methods.** 154 unselected children and adolescents (72 m, 82 f), aged 0.9÷19.3 yrs (mean age 8.7±4.5 yrs) at IDDM onset. Patients were diagnosed in our clinic and are controlled every 2 months. Thyroid function (FT3, FT4, TSH) and antithyroglobulin and antiperoxidase autoantibodies (TA) were checked in each subject at diagnosis of IDDM and subsequently every 6 months during the follow-up. **Results.** Patterns in subjects with positive TA, respectively at onset of IDDM (Group A) and during the follow-up (Group B), are shown in the table:

positive TA	n°	sex (m/f)	age at onset of IDDM	follow-up: diagnosis of ATD(*) (n°)	follow-up: neg TA	follow-up: pos TA	goiter (n°)	abnormal thyroid function
Group A	16	9/7	8.1±3.8	10 (62%)	4	2	7	5
Group B	21	5/16	7.1±4.8	5 (23%)	13	3	4	3

(*) $p < 0.05$

ATD (chronic thyroiditis) was diagnosed in 15 patients with IDDM out of 154 (9.7%). In 3 cases ATD was diagnosed before IDDM (1.5±1.5 yrs), in 5 at its onset and in 7 after its diagnosis (4.5±2.2 yrs). At diagnosis of ATD, 7 patients were euthyroid, 6 had subclinical and 1 clinical hypothyroidism, 1 had subclinical hyperthyroidism. 10/15 subjects with ATD had some goiter. Thyroid function was normal and goiter absent in 21 patients with positive TA only. 2 further subjects had high TSH levels and negative TA, no goiter, normal ecography, FT3 and FT4. **Conclusions.** There is a high prevalence of thyroid dysfunction in children and adolescents with type 1 diabetes mellitus (39/154=25%). Positive TA at onset of IDDM can be related to an eventual appearance of ATD, while during the follow-up, it could be a transitory serological marker of autoimmunity or the first sign of a poliglandular autoimmune disease. TA, at high title and long standing, require rigorous controls of thyroid function for revealing a precocious gland dysfunction and beginning, eventually, an appropriate therapy.

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THYROID ANTIBODIES IN CHILDREN WITH NEWLY DIAGNOSED TYPE 1 DIABETES

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The objective of this study was to estimate the prevalence of thyroid antibodies: antithyroglobulin antibodies (TG-Ab) and thyroid peroxidase antibodies (TPO-Ab), and their association with markers of autoimmune pancreatic damage: islet cell antibodies (ICA) and glutamic acid decarboxylase antibodies (GADA), in children with newly diagnosed type 1 diabetes mellitus.

Material and methods: 75 patients (39 female and 36 male) aged 9.9±4.3 years (from 2 to 18 years) with newly diagnosed type 1 diabetes, who were hospitalized in years 1996-1998 in the Department of Pediatrics of the Medical University of Lodz were included into the study. In all patients TG-Ab and TPO-Ab (immunoenzymatic method) as well as ICA (indirect immunofluorescence method) and GADA (indirect microimmunofluorescence method) were measured. In all patients the levels of free triiodothyronine (fT_3), free thyroxine (fT_4) and thyrotropin (TSH) were determined.

Results: In 10 patients (13.3%) elevated levels of TPO-Ab were found, and in 4 of them (5.3%) levels of TG-Ab were elevated, as well. At the moment of diabetes diagnosis ICA were positive in 78.9% and GADA in 74.3% of patients. The ICA levels did not differ significantly between the group with elevated and normal thyroid antibodies (mean value 60.5±69.8 JDF and 61.3±102.6 JDF respectively). The GADA levels were significantly higher in patients with elevated thyroid antibodies (mean value 101.3±66.0 AU, median value 110.0 AU) than in patients with normal thyroid antibodies (mean value 46.6±51.7 AU, median value 26.0 AU. $p<0.02$). In two girls with positive TPO-Ag hyperthyroidism in the course of Graves' disease was diagnosed. In one boy from this group, without any clinical symptoms of hypothyroidism, elevated TSH was found.

Conclusions: 1) In more than 13% of children with newly diagnosed type 1 diabetes the levels of thyroid antibodies (TPO-Ab and/or TG-Ab) are elevated, 2) There is a tendency to simultaneous occurrence of elevated thyroid antibodies and GADA, 3) Young patients with newly diagnosed type 1 diabetes, and particularly those of them with high GADA levels, should be screened for thyroid autoimmune process.

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Screening for coeliac disease in a paediatric diabetic population in Austria

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Diagnosis of unrecognised coeliac disease in patients with insulin-dependent diabetes mellitus is potentially important and prevalence rates of up to 10 % coeliac disease in diabetic patients have been reported.

A cohort of 400 Austrian children and adolescents with insulin-dependent diabetes mellitus (age range 3-22 yr.) was screened for coeliac disease using the IgA- anti-endomysial antibody test or in IgA deficient patients the IgG anti gliadin antibody test. Twelve patients (3 %) had increased anti endomysial antibody titres and two patients (0.5 %) with IgA deficiency had IgG antigliadin antibodies. All of them, with one exception due to refusal, underwent intestinal biopsy. Six patients had clear histologic evidence of coeliac disease (1.5 %), while three showed minor histological changes (0.8 %) and four had a normal mucosa (1.3 %). Only one child presented with mild clinical symptoms. There was no difference in the HbA1c level between antibody positive and negative cases and subsequent gluten free diet did not change the metabolic parameter.

Conclusion. The prevalence of clinically unrecognised „silent" coeliac disease is increased in Austrian diabetic children compared to the prevalence of symptomatic coeliac disease in the Austrian population (1.5 % vs. 0.33 %). But the prevalence of coeliac disease in diabetic children in Austria is distinctly lower than in several other countries. Screening with anti endomysial antibodies is recommended to identify diabetic children at risk, although the therapeutic benefit of dietary treatment in asymptotic „silent" cases has to be evaluated.

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GLYCEMIC CONTROL AND GROWTH BEFORE AND AFTER DIAGNOSIS OF COELIAC DISEASE IN CHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES

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Aims: To study the effects of undiagnosed coeliac disease (CD) on one hand and introduction of gluten-free diet on the other hand on the glycemic control and growth parameters in children and adolescents with type 1 diabetes. **Subjects and methods:** CD was found in 18 out of 776 subjects with type 1 diabetes (mean age 11.4 years, range 5.9 - 18.7 years) by screening for reticulin antibodies, confirmed by jejunal biopsy. We analyzed height standard deviation scores (HSDs), relative weights (% deviation from population median weight for height and sex) and levels of glycated hemoglobin A1 or A1c (GHbA1) at the diagnosis of CD and 1 year before and after that. Individual growth curves were analyzed from birth to the present time. **Results:** No significant change in GHbA1 levels was found preceding the diagnosis of CD. In subjects with a short duration of diabetes (≤3.5 years, n=8), a tendency towards deteriorating glycemic control after the diagnosis of CD was noticed (mean 19.8±32.6% increase in GHbA1, p=0.12). This probably was at least partially due to problems associated with the change of diet, because much smaller increase in GHbA1 was seen in this group during the 1 year period before diagnosis of CD (mean 3.2±16.1%, p=0.61). Failure in height and weight gain was found only in one subject, and it was improved by gluten-free diet. In the other subjects growth was not disturbed; mean HSDs (±SD) was 0.07 (±0.87) 1 year before diagnosis of CD, -0.02 (±0.88) at diagnosis of CD, and -0.08 (±0.87) 1 year after diagnosis of CD (p=0.47). Introduction of gluten-free diet led to an increase in mean relative weight from 4.3±18.1 to 8.2±15.4 % (p=0.02). There was an inverse correlation between the change in relative weight and the change in GHbA1 after the diagnosis of CD (r=–0.574, p=0.02). **Conclusions:** Undiagnosed CD is rarely associated with progressive deterioration of glycemic control or growth in children and adolescents with type 1 diabetes. Introduction of gluten-free diet tends to impair glycemic control in subjects with short duration of diabetes. We recommend careful counselling and follow-up for these subjects.

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SCREENING FOR CELIAC DISEASE IN CZECH DIABETIC CHILDREN

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Aims:

IDDM is often associated with other autoimmune disorders, among which celiac disease (CD) has especially high prevalence. The aim of the study was to develop a routine CD screening protocol for diabetic children, based on combination of genetic and immunological markers.

Material and methods:

341 patients with IDDM aged 1-17 were investigated for presence of anti-gliadin IgG and IgA antibodies in the first, and for endomysial antibodies (EmA) in the second step. In EmA positive individuals, enterobiopsy and the HLA-DQ, -DR genotyping was performed. 153 diabetic children without CD were HLA class II genotyped as a control group.

Results:

17 of 341 children (5.0%) were positive for EmA. Enterobiopsy and HLA typing were performed in 15 of them. Small bowel mucosa atrophy as a diagnostic sign of CD was found in 11 children (3.2% of the group). In 4 the enterobiopsy finding was normal, including activities of lactase and other investigated enzymes, and diagnosis of latent CD was concluded. All 15 children were positive either for DQA1*03-DQB1*0302, or DQA1*05-DQB1*0201, or both. 12 of 15 children with CD were DQA1*05-DQB1*0201 positive (conferring OR for CD 4.0 (CI 95% 1.1-15) against diabetic children without DQA1*05-DQB1*0201). 10 children were DQA1*03-DQB1*0302 positive, without significantly increased OR. 7 children carried HLA DQA1*03/05, DQB1*0201/0302, without significantly increased OR.

Conclusions:

Prevalence of celiac disease 5.0% in diabetic children emphasises the need of regular screening. The HLA DQA1*05-DQB1*0201, carried by 51% of Czech diabetic children without CD, was found to significantly increase the risk for CD. A routine screening protocol differentiated according to the HLA class II genotype is proposed.

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CELIAC SPRUE AND IDDM IN PREGNANCY

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The celiac disease (CD) is associated with various obstetric an gynaecological abnormalities and shows a higher prevalence in insulin dependent (IDDM) diabetic patients. The impaired metabolic milieu of diabetic pregnancy affects the development of the foetus so that whatever disease influences the diet and nutrients, may add a further damage to the conceptus and worsen the prognosis for the mother and foetus. Therefore we investigated 3 pregnant women affected by CD and IDDM and the presence of the Endomysial Antibodies (EMA) was studied in 63 IDDM pregnant women. In these women we evaluated: age of onset and duration of diabetes, insulin requirement, abortions, hypoglycaemic reaction, the newborn's time of delivery, any complication during pregnancy. The global prevalence of celiac disease (3 overt and 5 EMA positive) in our diabetic pregnant women (CD-IDDM) was 12%. In the CD-IDDM women as compared to EMA negative-IDDM women we observed longer duration of disease (23.4 ± 8.4 vs 14.2 ± 6.9 yrs; $p<0.02$) and younger age at the onset of diabetes (6.3±4.7 vs 14±6.7 yrs; $p<0.01$). In addition higher levels of blood glucose were found in CD+IDDM versus Ema-negative-IDDM during the first and second trimester (148±27 vs 124±29 mg/dl; I trim; $p<0.05$; 129 ±5 vs 121 ± 25 mg/dl; $p<0.05$ II trim. In CD-IDDM, severe (grade 3 and/or 4) hypoglycaemic reactions were increased (30% vs 9%; $p<0.05$). In addition we observed history of abortion and/or intrauterine death in about 50% in CD-IDDM vs 23% in the Ema-negative ($p<0.05$). These results suggest that clinicians need to be aware of this disease because it could have implications for the correct management of pregnancy in a high risk diabetic population.

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Nephropathy: Risk Factors

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PROGRESSION PROMOTERS AND TARGET BLOOD PRESSURE IN TYPE 1 DIABETIC PATIENTS WITH DIABETIC NEPHROPATHY

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Aim: To evaluate putative promoters of progression in Type 1 diabetic patients with diabetic nephropathy. **Material and methods:** All albuminuric Type 1 diabetic patients (n=304) followed at SDC for at least 3 years, (median(range)) 6.7 (3-20) years, who had at least 3 measurements (8 (3-31)) of glomerular filtration rate (GFR)(^{51}Cr-EDTA technique), were identified. GFR was measured yearly in 194 male/110 female patients, age 36 ± 11 (mean ± SD) years, diabetes duration 22 ± 8 years. 274 patients were treated with antihypertensive drugs, 180 predominantly with ACE inhibitors. **Results:** During the investigation period the decline in GFR (ΔGFR) was 4.1 ± 4.1 ml/min/year. A multivariate analysis revealed a significant correlation between the rate of decline in GFR and average values obtained during follow up of mean arterial blood pressure (MABP), HbA1c and albuminuria (R^2 adj = 0.27, $p<0.01$). No association with age, sex, retinopathy, smoking status, cholesterol, height, type of antihypertensive medication (ACEI vs non-ACEI) and ΔGFR was demonstrated. Patients were stratified in quintiles by average value of MABP and by HbA1c above and below the median during the investigation period:

	ΔGFR (ml/min/year), Mean(SE)				
MABP(mmHg)	90.8	98.0	102.0	105.5	112.0
HbA1c < 9.2%	1.4 (0.4)	1.5 (0.5)	3.6 (0.6)	4.4 (0.7)	6.0 (0.9)
HbA1c > 9.2%	3.3 (0.6)	3.5 (0.6)	4.6 (0.4)	6.1 (0.8)	7.5 (1.0)

Conclusions: Our study demonstrates, that arterial blood pressure, albuminuria and glycemic control act as progression promoters, and suggests a target MABP of approximately 94 mmHg. Remission of diabetic nephropathy, i.e. ΔGFR equal to natural ageing process (app. 1 ml/min/year), may be feasible when the treatment strategy combines strict blood pressure and glycemic control.

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INCIDENCE RATES OF ENDPOINTS IN TYPE 2 DIABETIC PATIENTS BY LEVEL OF SYSTOLIC BLOOD PRESSURE AND DURATION OF DIABETES

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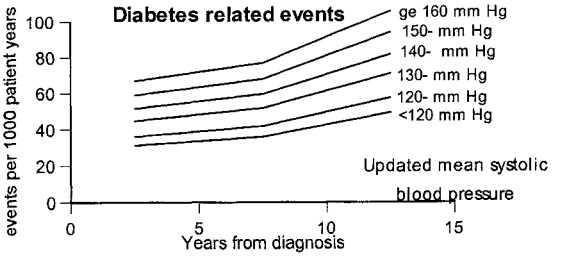
Aims To examine the event rates for different endpoints in the UKPDS by updated mean systolic blood pressure by time from diagnosis of Type 2 diabetes. **Methods** The UK Prospective Diabetes Study was a randomised controlled trial of therapies for blood glucose and blood pressure control, which ran in 23 centres in the UK between 1977 and 1997. From this study 4831 newly diagnosed Type 2 patients (mean age 53 years) who had data available for systolic blood pressure, age, race, gender were analysed in a Poisson regression model, with endpoints of any diabetes related endpoint, diabetes related death, all cause mortality, myocardial infarction, stroke, microvascular diasease,peripheral vasular disease and amputation. Mean updated systolic blood pressure was fitted in the model. **Results** For each endpoint the event rate increased monotonically with systolic blood pressure, and with duration of diabetes. **Conclusion** Treatment of elevated blood pressure has been shown to reduce the incidence of any diabetes related endpoint. The epidemiological evidence shows there may be potential to reduce the complications across the range of systolic blood pressure.



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CIRCULATING ADVANCED GLYCATION END PRODUCTS AS A MARKER OF LONG-TERM GLYCEMIC CONTROL AND DIABETIC NEPHROPATHY.

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Aims; Advanced glycation end products (AGE) play an important role in the pathogenesis of diabetic complications. Thus, evaluation of AGE may serve both for assessing glycemic control and the risk of diabetic complications. Materials and Methods; We have recently prepared polyclonal antibodies that recognized CML, pentosidine, and non-CML (non-pentosidine) AGE. Using these antibodies, we made ELISAs for detecting CML, pentosidine, and non-CML AGE. Serum levels of CML, pentosidine, and non-CML AGE were assessed in 22 Type 2 diabetic patients with microalbuminuria (Mi), 15 Type 2 diabetic patients with macroalbumiuria, (Ma), and 36 nondiabetic controls (N). Also, the mean fasting plasma glucose (FPG) level and HbA1c level, as well as CML, pentosidine, and non-CML AGE, were monitored monthly for 12 months in 18 Type 2 diabetic patients. Results; The strongest correlation was seen between HbA1c and the mean FPG for the previous month. However, the strongest correlation of the serum non-CML AGE or pentosidine levels was with the mean FPG over the previous 3 months (r=0.510, and r=0.352, respectively,). The levels of non-CML AGE and pentosidine, but not CML, increased with the progression of diabetic nephropathy (non-CML AGE; N: 19.3±6.8, Mi: 25.5±8.7, Ma: 39.3±16.4 U AGE/ml, p<0.01, Pentosidine; N: 198.8±52.6, Mi: 214.5±55.8, Ma: 266.2± 145.4 pmol/ml). Also, the % increase of the albumin excretion rate over the previous one year was significantly correlated with the non-CML AGE and pentosidine in levels over the past 3 months. Conclusions; These data suggested that measurement of circulating non-CML AGE and pentosidine may provide a longer-term index of the circulating glucose concentration compared with HbA1c. In addition, circulating non-CML AGE and pentosidine might be useful markers for assessing the risk of progression of diabetic nephropathy.

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RENAL FUNCTION IN NIDDM WITH CORONARY HEART DISEASE.

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Renal deficiency (RD) in NIDDM is,also, a cardio vascular risk factor. The aim of this work is the determination of renal function (RF) in NIDDM population with severe coronary heart disease (CHD).

In a prospective study, 40 consecutives NIDDM, 22 F/12M, with severe CHD (myocardial infarction, angioplasty,surgery) (C) were investigated and compared with 70 consecutives NIDDM without CHD (T).RD : creatinine clearance (clCR) ≤ 60 ml/ml and/or micro (mA)/macro (MA)-albuminuria (mMA) > 100 mg/24h is found in 82,5 % (n = 33) (C) VS 54 % (n = 38) (T) (P < 0,001) and is more important in C (P< 0,001) : CR (µmol/l) : 125 ± 78 vs 74 ± 25 et cl CR (ml/mn) :64 ± 33 VS 85 ± 26, mMA (%) : 73,5 VS 44 (mA : 50 % VS 38,6 % - NS - MA : 22,5 VS 5,7 % - p < 0,01)albuminuria (mg/24h) : 435 ± 774 VS 248 ± 502-NS. NIDDM with severe RD are mainly found in C (%) : CR ≥ 150 µmol/l : 15 VS 1,4 and mMA : 23 VS 5,7,while blood pressure level (mmHg) is highter : SBP : 151 ± 16 VS 134 ± 10 and pulse pressure (PP) : 68 ± 15 VS 58 ± 13 (DBP and MBP-NS).In C renal artery stenosis, (RAS) prevalence is important : 28 % (n = 11) VS 2,9 % (n = 2), as well as other severe macroangiopathies (SMAg Total % : 40 VS 10,7 - P < 0,01). Are also different (P < 0,01) C VS T : age (yr) : 70,8 ± 6,3 VS 67 ± 9,2, diabetes duration (yr) : 15,6 ± 9 VS 9,7 ± 5, sex ratio (F/M) : 1,2 VS 2,2, retinopathy prevalence (%) : 62,5 VS 41,4. RF of the only NIDDM with nephropathy (C- n= 32 VS T- n = 38) is more impaired in C (P < 0,01) : CR (µmol/l) : 139 ± 95 VS 82 ± 30, cl CR (ml/mn) : 58 ± 34 VS 79 ± 42, Ma (%) : 28 VS 10, while are highter SBP: 144 ± 14 VS 137 ± 12 and SMAg prevalence (44 VS 15 %) especially RAS : 34 % VS 5,3 %

Conclusion : In NIDDM with severe coronaropathy, renal deficiency is frequent (83%),important, and often associated with other severe macro-angiopathy, especially renal artery stenosis (28%). These results suggest to look at renal arteries during coronarography in this population.

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FACTORS INFLUENCING PROTEINURIA IN PATIENTS WITH TYPE 2 DIABETES

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Diabetic nephropathy ensues as a complication of both Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetes mellitus. In Caucasians the prevalence of nephropathy, assessed on the basis of proteinuria, ranges between 12% and 16% of diabetics.The aim of the study was to estimate both the prevalence and incidence of proteinuria, and to find factors related to it during long-term follow-up investigation of Type 2 diabetes. In 4420 patients (1990 males and 2430 females) aged 30-68 years, with diabetes of 1-10 years' duration, proteinuria was found in 29% persons. Its presence was related to poor metabolic control of diabetes, arterial hypertension, and ecg-revealed cardiac ischaemia. After 16 years of follow-up (16-26 years of duration of diabetes), the prevalence of proteinuria was 32%, with the risk correlates identical to those at the initial investigation. In patients with longest duration of diabetes (22-32 years) proteinuria amounted to 53%. The death experience of the cohort revealed that initial proteinuria >300 mg/dl reduced survival by 90%, whereas its level ≤50 mg/dl allowed 75% of patients to survive. The data revealed a higher proteinuria in patients with Type 2 diabetes than is usually reported in other investigations - and show that its presence was similar to the levels found in Type 1 diabetes. It was shown that the presence of heavy proteinuria (≥50 mg/dl) increased with duration of the diabetes. It was related to the risk of ischaemic heart disease, together contributing to greatly reduced chances for survival.

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CARDIAC DEATH IN JAPANESE TYPE 2 DIABETIC PATIENTS DURING END-STAGE RENAL DISEASE THERAPY

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Aims: The excess mortality in diabetic end-stage renal disease (ESRD) is mainly due to cardiovascular events. The objective of this study was to determine prevalence and prognosis of cardiac disease during ESRD therapy and to detect risk factors of cardiac death in diabetic patients entering dialysis. **Materials and Methods:** An inception cohort of 243 consecutive Japanese Type 2 diabetic patients with ESRD had echocardiography and clinical assessment performed at the start of dialysis therapy. We followed prospectively 227 patients (159 men, mean age 58 (SD:10) years), who survived 6 months after inception of dialysis for a median of 48 months (range:6-117). Main outcome measure was cardiac death. **Results:** At baseline, 53 patients (23%) had clinical manifestation of cardiac diseases (coronary artery disease, valvular failure, hypertrophic cardiomyopathy). In addition, 155 (89%) patients had left ventricular hypertrophy in patients without clinical cardiac disease at the start of dialysis. During follow-up 109 patients died, and cardiac death was the highest event of mortality (40%) in this cohort. Cardiac death occurred more frequently in patients with clinical cardiac diseases (relative risk 3.98, p<0.05) at baseline. In Cox's proportional hazard model, factors affecting cardiac death before 48 months (median survival) were: fractional shortening (hazard ratio 0.93, 95% CI: 0.88 to 0.98), HbA_{1c} (1.47, 1.11 to 1.94), age (1.07, 1.01 to 1.12), and male gender (2.73, 0.96 to 7.75) after adjustment for body mass index, known diabetic duration, mean arterial blood pressure, left ventricular diastolic dimension, wall thickness, serum albumin, and hematocrit. **Conclusions:** Clinical and echocardiographic cardiac diseases, present at start of ESRD therapy, predict cardiac death in Japanese Type 2 diabetic patients. Decreased systolic function, metabolic control, and male gender are associated with high risks of early cardiac death during dialysis.

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ANALYSIS OF RISK FACTORS FOR RISE IN SERUM CREATININE IN TYPE 2 DIABETES WITH NEPHROPATHY

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【AIM】 To evaluate the risk factors for rise in serum creatinine (S-Cr) in type 2 diabetic patients with nephropathy. 【METHOD】 The subjects were 14 diabetic patients with overt proteinuria who had a S-Cr value of 1.5 mg/dl at the start of the study. They were divided into 2 groups according to the level of S-Cr at the third year of the study; 4 patients had a S-Cr level under 1.6 mg/dl (NC group), and in 10 patients the level was over 1.7 mg/dl (C group). In 2 groups blood pressure (BP), HbA1c, lipid, hematocrit (Ht) and serum albumin (Alb) were compared over a 3 year period. 【RESULTS】 There was no significant differences in age, sex, duration of diabetes, method of diabetic treatment, and kind of antihypertensive and antilipidemic agent between the 2 groups. In the C group, systolic BP in the third year was 149.4±20.9 mmHg, which was higher than the NC group (139.0±16.5 mmHg). There was a significant decrease in systolic BP during the 3 year period in all patients in the NC group, whereas 5 patients in the C group showed elevated systolic BP ($p<0.01$). The HbA1c level at one year before the investigation was higher in the C group (9.3 ± 1.3%) than the NC group (7.1 ± 0.6%). Triglyceride (TG) levels in the third year were significantly higher in the C group (347.9 ± 297.4mg/dl) than the NC group (237.4 ± 86.7mg/dl) ($p<0.05$). In multiple regression analysis, the only independent factor associated with the rise in S-Cr was HbA1c level. 【CONCLUSION】 Diabetic control, systolic BP and TG level appeared to influence deterioration in renal function in type 2 diabetes with nephropathy, with diabetic control being the most important factor.

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SODIUM-LITHIUM COUNTERTRANSPORT IS INCREASED IN NORMO-ALBUMINURIC TYPE 1 DIABETES BUT IS NOT ASSOCIATED WITH OTHER RISK FACTORS FOR MICROANGIOPATHY

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Aims: Increased sodium-lithium countertransport (Na-Li CT) activity has been reported to be associated with diabetic nephropathy. The mechanism underlying this association is not fully elucidated. Na-Li CT is a genetic marker of esential hypertension, linked to insulin-resistance and may reflect Na-H exchange activity. We studied the kinetic parameters of Na-Li CT activity in 53 normoalbuminuric type 1 diabetic patients (DP) and 45 matched control subjects (C). Other risk-factors for microangiopathy are studied and correlated with Na-Li CT activity. **Methods:** We measured Na-Li CT activity (maximum velocity (V_{max}) and sodium affinity (K_m)) according to the "Canessa" method. Endothelial function was assessed by infusion of acetylcholine. Blood samples were collected for HbA1c, glucose, insulin and lipids. Blood pressure was measured intra-arterially. Renal hemodynamics were measured by inulin/PAH clearance. Plasma volume was measured by ^{131}I-albumin. Outcomes were evaluated in DP by comparing the highest and lowest quartile of V_{max} and K_m. **Results:** V_{max} was increased in DP (779±265 mmol Li/hr/l and 630±236 in C, $p<0.01$). K_m was decreased in DP (64±16 mmol/l vs 76±27 in C, $p=0.01$).

	controls	DP V_{max} high	DP V_{max} low	DP K_m high	DP K_m low
number	45	13	13	13	13
V_{max}	630±236	1140±152	467±55	857±258	558±240
K_m	76±27	68±10	50±16	84±10	44±7

There were no differences between the highest and lowest quartiles of V_{max} or K_m with respect to blood pressure, renal hemodynamics, AER, plasma volume, endothelial function, HbA1c, glucose, insulin and lipid profile. **Conclusion**: Na-Li CT is increased in uncomplicated type 1 diabetes and characterised by an increase in V_{max} and a decrease in K_m. The increase in Na-Li CT nor the increase in sodium affinity is associated with changes in endothelial function, blood pressure, renal hemodynamics or plasma volume. Na-Li CT seems to be an independent risk marker of diabetic nephropathy.

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SERUM SIALIC ACID CONCENTRATION IN YOUNG INSULIN DEPENDENT DIABETES MELLITUS PATIENTS

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Aims: Elevated concentrations of serum sialic acid, a potent cardiovascular risk factor in general population, had been found in adults with IDDM especially in albuminuric patients. The aim of the study was to estimate the serum sialic acid concentration in young patients with IDDM. **Materials and Methods:** We studied 54 persons at the age 15-24 years: 21 non-diabetic controls, 32 IDDM patients with normoalbuminuria and 12 with incipient nephropathy (UAE 20-200μg/min) matched for sex, age and body mass index. Duration of diabetes was 11.5±3.7 years. Fasting blood samples were taken for sialic acid (colorimetric assay), cholesterol, HDL and LDL cholesterol, trigliceride, creatinine, glycated haemoglobin, and plasma fibrinogen and erythrocyte sedimentation rate. **Results:** Microalbuminuric patients had a higher serum sialic acid concentration (mg/ml) than non-diabetic controls (0.61± 0.16 vs. 0.5±0.07; $p<0.01$) and normoalbuminuric patients (0.61± 0.16 vs. 0.49±0.14; $p<0.05$). There was no significant difference between controls and normoalbuminuric patients. In the microalbuminuric group sialic acid concentration correlated significantly with HbA1c ($r=0.6$; $p<0.05$) and cholesterol ($r=0.6$; $p<0.05$). Comparison between paired group showed that serum trigliceride and cholesterol were significantly higher in group with microalbuminuria compared with the normoalbuminuric patients and controls. Retrospectively we measured the serum sialic acid concentration in frozen 4 years earlier serum of 21 examined diabetic patients who were then normoalbuminuric. 11 patients who became microalbuminuric had then higher level of sialic acid concentration than 10 patients who remained normoalbuminuric (1.01±0.4 vs. 0.63±0.2; $p<0.05$). **Conclusion:** The observations suggest that the serum sialic acid concentration is elevated in diabetic patients with microalbuminuria. An increased sialic acid concentration may be predictive for onset of microalbuminuria.

PS 84
Nephropathy: Gene Polymorphisms

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LACK OF CONTRIBUTION OF TWO ENDOTHELIAL NITRIC-OXIDE SYNTHASE (eNOS) GENE POLYMORPHISMS TO DIABETIC NEPHROPATHY IN TYPE 1 DIABETES.

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Aim : To study the relationship between diabetic nephropathy (DN) and two eNOS gene polymorphisms : a 27-bp repeat in intron 4 (eNOS 4a/4b) and a point mutation in exon 7 (Glu298Asp), we used two different approaches: a case-control study and a prospective follow-up study. **Patients and methods :** *Case-control study*: we included 493 type 1 diabetic patients with proliferative retinopathy (GENEDIAB Study). This group was made of 157 patients with no DN (controls) and 336 with various stages of DN (cases): 104 with incipient (I. DN), 126 with established (E DN) and 106 with advanced (A. DN) diabetic nephropathy. *Follow-up study*: we enrolled 298 type 1 diabetic patients attending our clinic. The main outcome was the occurence of a renal event defined as the progression from one stage of DN to a higher one. Time to progression curves were calculated using the Kaplan-Meier estimate, and Mantel-Cox logrank test was used for comparison of renal event-free survival between groups. *Genotyping*: eNOS 4a/4b was determined by PCR and the Glu298Asp by PCR and restriction with *BanII* and *MboI*. **Results :** *Case-control study*: there was no difference in the distributions of the polymorphisms between the different DN sub-groups (χ^2 for trend = 0.06 and 0.02, $p = 0.81$ and $p=0.90$, for eNOS4a/b and Glu298Asp respectively). *Follow-up study*: at baseline, 251 patients (81 %) had no DN, 35 (11%) had I. DN, 18 (6%) E DN, and 6 (2%) A DN. Median duration of follow-up was 6 years (range: 2-9). We fund no difference for renal event-free survival according to eNOS 4a/4b (n= 295) or Glu298Asp polymorphisms (logrank $\chi^2 = 0.45$, and 2.62, $p = 0.80$ and $p = 0.27$ respectively).

	Case-control study n (%)				Follow-up study n (%)
	No DN	I. DN	E. DN	A. DN	Progression
NOS4 bb	117 (75.0)	78 (75.0)	91 (72.8)	81 (77.1)	35/219 (16.0)
ba	34 (21.8)	25 (24.0)	32 (25.6)	21 (20.0)	14/69 (20.3)
aa	5 (3.2)	1 (1.0)	2 (1.6)	3 (2.9)	1/7 (14.3)
NOS7 Glu/Glu	64 (40.8)	46 (44.2)	50 (39.7)	48 (45.3)	21/118 (17.7)
Glu/Asp	71 (45.2)	41 (39.4)	52 (41.3)	43 (40.6)	25/132 (18.9)
Asp/Asp	22 (14.0)	17 (16.3)	24 (19.0)	15 (14.2)	4/48 (8.3)

Conclusion: These results do not support the hypothesis that the eNOS gene polymorphisms contribute to susceptibility of DN in type 1 diabetes.

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A HUMAN INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS) PENTA-REPEAT PROMOTER POLYMORPHISM CONFERS LOW RISK OF DIABETIC NEPHROPATHY IN IDDM

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In the pathogenesis of diabetic nephropathy nitric oxide (NO) has been proposed to induce glomerular hyperfiltration characteristic for early stage of diabetic nephropathy. In the later stages of diabetic nephropathy, endothelial damage due to the accumulation of advanced glucosylation end-products (AGEs) may inactivate NO with a subsequent decrease in glomerular function. iNOS has been identified in glomerular mesangial cells, vascular smooth muscle cells and vascular endothelial cells. Recently, a penta-repeat polymorphism $(CCTTT)_n$ within the human iNOS promoter was identified. One of the alleles (14 repeats, A14) was suggested to confer low risk of diabetic retinopathy in a mixed NIDDM and IDDM population. Furthermore, in a promoter activity assay the same allele correlated with an increased promoter activity as compared with other alleles. In an assay using radioactive PCR amplification of the polymorphism, followed by PAGE, we tested for this polymorphism in a large IDDM population with overt diabetic nephropathy (n=358; M/F: 213/145; UAE: 614 (10-14.545 mg/24hrs.); duration: 28 ± 8 years) and in a matched IDDM population with persistent normoalbuminuria (n=193; M/F: 119/74; UAE: 10 (1-30 mg/24hrs.); duration: 27 ± 8 years). In total 10 alleles were identified. No significant difference in distribution of alleles was observed comparing IDDM patients with or without diabetic nephropathy (Chi-square: 14.51, df: 9; p=0,11). The A14 allele frequency was significantly lower in the nephropathy group, 0.05 vs 0.09 in patients with normoalbuminuria (Chi-square: 6.00, df: 1; p=0,01). The OR for nephropathy in IDDM patients carrying the A14 allele is 0.54 (95% CI: 0.32 - 0.89). Comparing the overall allele distribution and the frequency of A14 in IDDM patient with or without nephropathy stratified for status of retinopathy (nihil, simplex, proliferative) no differences were observed. In conclusion, the A14 allele in the penta-repeat polymorphism of the human iNOS promoter region is associated with low risk of diabetic nephropathy in Caucasian IDDM patients. We could not confirm previous association between this polymorphism and another microvascular complication: diabetic retinopathy

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Endothelin-1 (ET-1) gene Bsl I polymorphism is associated with the development of diabetic nephropathy in patients with type 2 diabetes

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Results of studies on the role of endothelins in the pathogenesis of essential hypertension are conflicting. On the other hand, endothelin receptor agonists seem to ameliorate proteinuria in animal models of diabetic kidney disease. Thus, polymorphisms in the "endothelin system" (endothelins and endothelin receptors) are plausible candidates for the genetic component of the predisposition to diabetic nephropathy.

The **aim** of this study was to investigate the association between ET-1 gene polymorphism and nephropathy in type 2 diabetic patients. **Material and methods.** The protocol included 373 unrelated type 2 diabetic subjects selected from a group of 941 patients. The group consisted of subjects with renal status established according to albumin to creatinine ratio. Patients were divided into 3 groups. 112 (all available samples) subjects with proteinuria, and random samples of normoalbuminuric and microalbuminuric patients, n=135 and n=126, respectively (normoalbuminuric patients with known diabetes duration of at least 10 years formed the control group). All patients were Caucasians, inhabitants of the same city and attendants of the same diabetic clinic at the time of examination. DNA was obtained from peripheral blood leukocytes. The polymorphism was determined by polymerase chain reaction and restriction fragment length protocol using Bsl I endonuclease.

Genotype	Normoalbuminuria	Microalbuminuria	Proteinuria
	n=135	n=126	n=112
1.1	63 (46.67 %)	63 (50.0 %)	56 (50 %)
1.2	61 (45.18 %)	54 (42.86 %)	37 (33,04 %)
2.2	11 (8.15 %)	9 (7.14 %)	19 (16,96 %)

Results. We observe significant difference in genotype distribution between the investigated groups: the 2.2 genotype was found to be significantly more frequent among proteinuric cases, when compared to patients with microalbuminuria, as well as to normoalbuminuric controls (chi^2 test $p<0.05$), but with no difference between normo- and microalbuminuric patients. **Conclusions.** Results may suggest the role of ET-1 polymorphism in the predisposition to more advanced stages of nephropathy (clinically manifested as overt proteinuria) in type 2 diabetic patients.

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POLYMORPHISMS IN THE GENES ENCODING FOR BRADYKININ RECEPTORS DO NOT CONTRIBUTE TO THE INCREASED RISK OF NEPHROPATHY IN TYPE 2 DIABETIC PATIENTS

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Aim: Kinins acting via specific receptors – B1R and B2R constitute a vascular system regulating blood pressure and local hemodynamics, and modulate tissue inflammatory response and cell proliferation. Recent findings suggested a nephroprotective role of kinin peptides; it was also pointed, that beneficial effect of ACE-inhibitors is in part a result of their ability to potentiate kinin activity. Case-control, as well as family based comparisons have shown a non-specific, protective effect of B1R gene promoter G-699C polymorphism, and possibly, B2R exon 2 C181T polymorphism against the development of chronic renal failure in the course of glomerular or tubulo-interstitial diseases. However, the role of these polymorphisms in the predisposition to different stages of diabetic nephropathy remains to be determined. Meterials and Methods. In this study, B1R gene promoter G-699C and B2R exon 2 C181T polymorphisms were genotyped in type 2 diabetic patients with renal status determined according to measurements of urinary albumin/creatinine ratio, serum creatinine concentration and a review of medical records: 153 patients with microalbuminuria, 109 with overt nephropathy (among them 49 with raised serum creatinine), and a control group of 161 patients with normoalbuminuria and diabetes duration ≥ 10 yrs. Results: We observed no significant differences (χ^2 test) in the distributions of the examined polymorphisms between the study groups When patients with raised serum creatinine levels were analysed separately, surprisingly, a tendency towards higher frequency of B1R GC and CC 'protective' genotypes was observed (34.7%, p=0.06 vs. normoalbuminuria), most likely due to a random distortion in a small sample size.

	normo	micro	overt
B1R promoter G-699C	%	%	%
GG	78 3	78.4	74.3
GC+CC 'protective'	21.7	21.6	25.7
B2R exon 2 C181T	%	%	%
CC	87 1	83.9	88.2
CT+TT 'protective'	12 9	16.1	11.8

Conclusions: These data may suggest, that kallikrein – kinin system, and bradykinin receptor polymorphisms are not one of the key mechanisms leading to renal damage in the course of type 2 diabetes.

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POLYMORPHISM IN THE 5'-END OF THE ALDOSE REDUCTASE GENE IN DIABETIC ADOLESCENTS AND YOUNG ADULTS WITH MICROALBUMINURIA.

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Aim: to investigate the genetic risk factor for the development of diabetic nephropathy early in the course of type 1 diabetes. **Materials and Methods:** DNA was isolated from 63 patients aged 13.5-27 years with type 1 diabetes from 8.3-21.5 years and 47 healthy controls. $(GT)_n$ dinucleotide repeat polymorphic marker in the 5'-end of the aldose reductase gene was determined using PCR based acrylamide gel electrophoresis genotyping. **Results:** 9 allele were identified in the group of patients (Z-10 to Z+6). A higher frequency of the Z-2 allele was found in the group with persistent microalbuminuria (PMA) (n=8) compared with two other groups of patients with normoalbuminuria (NA) (n=41) and intermittent microalbuminuria (IMA) (n=14), and with controls (C), (PMA-62.5%, IMA-39.3%, NA-43.9%, C-18.1%). The PMA group had also the increased frequency of Z-2/Z-2 genotype in comparison with the other groups (PMA-37.5%, NA-2.4%, IMA-0%, C-0%). Three adolescents with microalbuminuria of early onset (below 10 years of diabetes) and rapid progression were homozygotes for Z-2 allele. **Conclusions:** These preliminary results suggest that aldose reductase gene may be involved in the susceptibility to early development of diabetic nephropathy.

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POLYMORPHISM *SCAI* OF THE ATRIAL NATRIURETIC PEPTIDE GENE, DIABETIC NEPHROPATHY AND HYPERTENSION IN TYPE 1 DIABETES.

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Aim: Development of diabetic nephropathy (DN) seems to be partially under genetic control. Atrial natriuretic peptide (ANF, jointly affects kidney function and blood pressure homeostasis, and might be involved in the susceptibility to DN and hypertension (HT). We evaluated the relationship between the *ScaI* polymorphism of the *ANP* gene and DN and HT in type 1 diabetic patients. Materials and Methods: A fragment of 133 bp of the hANP gene was amplified by polymerase chain reaction (primers: sense, 2158-2185; antisense, 2330-2349 of published sequence). Allele frequencies of this polymorphism were studied by RFLP analysis with *ScaI* enzyme. Results: Of 635 type 1 patients, 116 had microalbuminuria (μA) and 115 had established DN; 58 healthy controls (C) were included. In the whole type 1 cohort, the wild (A^2) and mutated (A^1) alleles had frequencies of 0.73 and 0.27, and genotypes were in Hardy-Weinberg equilibrium. Allele frequencies (A^2 *vs* A^1) were 0.82 and 0.18 in normoalbuminuric patients (NA); 0.89 and 0.11 in μA; 0.94 and 0.06 in DN, and 0.80 and 0.20 in C ($p<0.0001$ for the difference between DN and the other groups). This difference persisted when the analysis was restricted to the patients with diabetes duration >15 years ($p=0.001$). Moreover, alleles A^2 and A^1 were 0.82 and 0.18 in normotensive and 0.91 and 0.09 in hypertensive patients ($p=0.0001$). While no differences were found in the distribution of the two alleles according to HT in subjects with DN, A^1 allele frequencies were 0.07 and 0.13 in NA patients with and without HT, respectively ($p<0.004$). These results were confirmed in NA with long diabetes duration. Conclusions: In type 1 diabetic patients, the A^1 allele of *ANP gene* is significantly less frequent in patients with DN. Moreover, in NA subjects the A^1 allele is less frequent in the presence of HT. Thus, this polymorphism may play a role in protecting type 1 diabetic patients against kidney disease and hypertension.

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MITOCHONDRIAL GENOTYPE SUSCEPTIBLE TO TYPE 1 DIABETES PREDISPOSES PATIENTS TO DEVELOP DIABETIC NEPHROPATHY

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Oxidative stress by infiltrating inflammatory cells plays an essential role in the destruction of pancreatic β cells in type 1 diabetes mellitus and continuous hyperglycemia induces generation of reactive oxygen species leading to diabetic vascular complications. Mt5178A genotype was reported as one of the longevity genotypes and is suggested to be more resistant than Mt5178C genotype against oxygen radicals. To examine whether or not this polymorphism influences development of type 1 or type 2 diabetes and its diabetic complications, we analyzed the frequencies of Mt5178A/Mt5178C by PCR-RFLP with *Alu* I in 385 type 1 diabetic patients selected randomly among follow-up patient and 687 type 2 diabetic patients recruited randomly from out-patients for 2 days in our Diabetes Center. The frequency of Mt5178C in 385 type 1 patients was significantly higher than that of 163 healthy control who had normal glucose and lipid metabolism ($p=0.03$, Odds ratio 1.52, 95%CI 1.04-2.22). In the 116 type 1 patients with diabetic duration of more than 20 years, the Mt5178C frequency in Nephropathy group (UAE : first morning urinary albumin excretion >12 mg/gCr) was statistically higher than that in No Nephropathy group (UAE≦12 mg/gCr) ($p=0.004$, Odds ratio 3.25, 95%CI 1.49-7.09). There were no differences in the Mt5178A/Mt5178C frequency between three retinopathy stages in these patients. On the other hand, the frequency of Mt5178C in type 2 patients was not significantly higher than healthy control. The life table analysis with respect to the onset age of type 2 patients showed no significant difference between 2 genotype groups. The analysis with respect to development of proliferative retinopathy and persistent proteinuria showed that the frequency of Mt5178C increased more than that of Mt5178A within around 15 year diabetic duration, which was not significant. These results suggest that Mt5178C is susceptible to type 1 diabetes and IDDM patients with Mt5178C are more predisposed to diabetic nephropathy than those with Mt5178A.

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Role of PC-1 gene polymorphism (K121Q) in the progression of renal disease in type 1 diabetic patients.

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Environmental and genetic factors are both involved in the progression of diabetic nephropathy (DN). Insulin resistance is associated with diabetic nephropathy and is present in non-diabetic parents of type 1 diabetic patients. PC-1 glycoprotein interferes with insulin action and we have recently described a polymorphism of its gene (K121Q) which is associated with insulin resistance in the general population. Because of this observation, we have examined the relationship between this novel polymorphism of PC-1 and progression of DN in type 1 diabetic patients. In seventy-seven type 1 diabetic patients with DN followed prospectively for an average of 7 yrs (range: 2,5-19) with six monthly measurements of creatinine clearance (CrCl)(Cockroft formula) we compared the rate of CrCl decline according to the PC-1 genotype (KK n=55 vs KQ+QQ n=22). Gene polymorphisms were determined by PCR. The average rate of decline of CrCl was 5.8±4 ml/min/yrs. Patients with the genotype PC-1 KK showed a slower rate of disease progression (KK: 5.1±5 vs KQ+QQ: 7.3±4 ml/min/year $p<0.05$). The effect of the PC-1 genotype was independent by known environmental promoters of progression of DN such as blood glucose and blood pressure control. In conclusion the candidate gene examined in this study is associated with the progression of DN. PC-1 genotyping may be, therefore, useful for identifying type 1 diabetic patients at risk of rapidly development of end stage renal failure.

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Nephropathy: Morphology and ACE genes

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GLOMERULAR ULTRASTRUCTURE IN TYPE 2 DIABETES.

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Ultrastructural measures of glomerular lesions are related to renal dysfunction in type 1 diabetes (D1) but much less is known on these relationships in type 2 diabetes (D2). Aims: to evaluate glomerular structure in D2. Methods: we performed kidney biopsy and renal function studies in 73 D2 pts: 49 had albumin excretion rate (AER) 20-200 (microalbuminuria-MA) and 24 >200 (proteinuria-P). The structural data were compared to those of 27 normal age matched kidney donors (C) and 64 D1: 22 had MA and 42 P. Electron microscopic morphometric analysis was used to estimate glomerular basement membrane (GBM) width and mesangial [Vv(mes/ glom)], matrix [Vv(MM/glom)] and cell [Vv(MC/glom)] volume fractions. Results: Known D2 duration was longer in P than MA (16±6 vs 11±6 yrs, p<.005) while age, HbA1c (8.4±1.6 in MA and 8.9±1.5% in P) and GFR (101±29 ml/min/1.73 m^2 in MA and 92±35 in P) were similar in the 2 groups. All structural parameters were abnormal in MA [GBM width: 432±102 nm, C: 310±38, p<.001; Vv(mes/glom): .26±.06, C: .19±.03, p<.001; Vv(MM/glom): .13±.04, C:.09±.02, p<.01, except for Vv(MC/glom): .09±.03, C: .09±.02, ns]. Glomerular lesions were more advanced in P than in MA [GBM width: 557±120, p<.001; Vv(mes/glom): .33±.10, p<.002; Vv(MM/glom): .17±.06, p<.005; Vv(MC/glom): .11±.04, p<.05]. Log AER was directly related to GBM width (r.40, p<.001), Vv(mes/glom) (r.36, p<.005), Vv(MM/glom) (r.34, p<.005), Vv(MC/glom) (r.23, p<.05). GFR was inversely related to Vv(mes/glom) (r.49, p<.001), Vv(MM/glom) (r.46, p<.001), Vv(MC/glom) (r.38, p<.001) but not to GBM. Although these structural/functional relationships were significant in D2 they were imprecise and less strong than in D1. This was explained by a significant proportion of D2 pts with MA and P having normal glomerular parameters: indeed more D2 than D1 pts with MA had normal Vv(mes/glom) values [22/49 (49%) D2 vs 1/22 (4.5%), Chi-square, p<.001]. Similarly more D2 than D1 pts with P had normal Vv(mes/glom) [7/24 (29%) in D2 vs 0/42 in D1, Chi-square p<.001). Conclusions: 1) All structural parametes are related to AER while only Vv(mes/glom) and its components are related to GFR. Thus, in D2 as in D1 mesangial expansion is a crucial structural change, leading to loss of renal function. 2) Glomerular lesions are less advanced in D2 than in D1 pts and a substantial number of D2 pts have normal glomerular structure despite MA or P. This is in agreement with our light microscopy studies demonstrating that a proportion of D2 pts have more advanced tubulo-interstitial and vascular than glomerular lesions.

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RENAL BIOPSY IN PROTEINURIC TYPE-2 DIABETIC PATIENTS

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Background: In type2 diabetes (DM), detection of non-diabetic glomerular disease (NDGD) is important from therapeutic point of view. **Aim:** To identify the proteinuric type-2 DM patients at greater risk of having NDGD and thus requiring renal biopsy. **Materials & Methods:** Patients with Type-2 DM irrespective of duration, proteinuria >=1.5 gm/24 hr. without urinary infection or heart failure, preserved and symmetrical kidney size with either "absense of diabetic retinopathy (DR)" or "presence of erythrocyturia" were considered. Total 46 patients (m=25, f=21) of mean (±SD) age 54.4±8.04 years with known diabetic duration of 9.17± 6.26 years had ultrasound guided renal biopsy during the period of July 1995 to June 1997. **Results:** Clinico-morphological associations are shown below:

Biopsy		D.Retinopathy		Erythrocyturia		Hypertension		Proteinuria
	n	*-ve*	*+ve*	*-ve*	*+ve*	*-ve*	*+ve*	*(gm/day)*
DN	19	3	16	3	16	3	16	6.9± 2.1
NDGD	27	22	5	14	13	3	24	4.9±2.9

Positive predictive value (PPV), negative predictive value (NPV), sensitivity (SEN) and specificity (SPEC) of *"absence of DR"* (diagnostic accuracy 82.6%, p<0.001) and *"<=7yr of diabetic duration"* (diagnostic accuracy 71.73%, p=0.004) for NDGD are shown below:

	Criteria "D.Retinopathy -ve"			*Criteria "Duration of DM<=7yr"*		
	For all patients	male	female	For all patients	male	female
PPV (%)	88.0	66.6	100.0	85.0	83.3	85.7
NPV (%)	76.2	81.3	60.0	61.5	78.9	14.3
SEN (%)	81.5	66.6	88.8	62.9	55.5	66.6
SPEC (%)	84.2	81.2	100.0	84.2	93.7	33.3

Logistic regression also confirmed the independent association of "absence of DR" (p=0.0006) and appearance of proteinuria "within 7 years of diabetic duration" with presence of NDGD in Type-II DM. **Conclusion:** Renal biopsy is strongly recommended in type-2 DM if proteinuria appears without appearance of diabetic retinopathy or within 7 years of known diabetes onset.

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THE CAUSES OF ALBUMINURIA IN CAUCASIAN NIDDM PATIENTS WITHOUT DIABETIC RETINOPATHY

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Aims: To evaluate the causes of albuminuria in NIDDM patients without retinopathy.

Materials and Methods: 347 consecutive NIDDM patients with persistent albuminuria (> 300 mg/24 h) was recorded. Fundus photo (80%) and ophthalmoscopy was performed. 93 (27%) had no retinopathy and a kidney biopsy was performed in 52 (56%) of these patients. The biopsies were evaluated by light, electron and immunofluorescent microscopy by three masked nephropathologists. Patients were not referred for a kidney biopsy if; contraindications (n = 13, 14%), or albuminuria < 1 g/24 h (n = 15, 16%) or if the age was > 65 years (n = 7, 8%). The remaining 6 (6%) did not want to participate.

Results: The biopsy revealed diffuse and/or nodular diabetic glomerulosclerosis in 69% of the patients (28 male/7 female), while the remaining 31% (13 male/3 female) had non-diabetic glomerulopathies, such as; different types of glomerulonephritis (n = 7, 13%) and normal or near normal glomerular structure (n = 9, 18%). Baseline was defined as the time of first GFR measurement. The interval between known onset of persistent albuminuria and first GFR measurement was 1 (0-6) vs 1.5 (0-6) years, median (range) in patients with and without diabetic glomerulosclerosis, respectively. At baseline we found no significant difference in sex, age 56 (8) vs 53 (10) years, mean (SD), body mass index 30 (4) vs 31 (8) kg/m^2, known duration of diabetes 6 (6) vs 4 (3) years, GFR 95 (29) vs 89 (31) ml/min/1.73 m^2 , albuminuria 1304 (169-4731) vs 1050 (181-5176) mg/24 h, blood pressure 150/87 (16)/(9) vs 145/89 (16)/(9) mm Hg, prevalence of hypertension 89 vs 100 %, haemoglobin A_{1c} 8.2 (1.6) vs 9.0 (2.5) % and serum total cholesterol 7.1 (2.4) vs 6.3 (1.6) mmol/l, between patients with and without diabetic glomerulosclerosis.

Conclusion: Albuminuric NIDDM patients without retinopathy require further evaluation, that is, kidney biopsy, since the chance for a non-diabetic glomerulopathy is approximately 30%. A separation between the two main types of glomerular lesion was not possible based on demographic or clinical nor laboratory data.

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THE ROLE OF MACROVASCULAR DISEASE IN THE PATHOGENESIS OF RENAL DAMAGE IN TYPE 2 DIABETIC PATIENTS.

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Type 2 diabetic patients (D) with microalbuminuria (MA) have heterogeneous renal structure. Only ~30% of MA D have typical diabetic nephropathology (CII), while ~30% have near normal structure (CI) or atypical patterns of injury (CIII) with more severe tubulo-interstitial and/or vascular than glomerular changes (~30%). It is possible that tubulo-interstitial lesions and small kidney size are due to macroangiopathy. Aims: to evaluate the role of macroangiopathy in determining renal lesions. Methods: we studied 41 D 56±7 yrs old (mean±SD) with serum creatinine <2mg/dl and abnormal albumin excretion rate (AER+) (20-6628µg/min) and 9 normal subjects (N) 43±16 yrs. old In all pts we evaluated: AER, glomerular filtration rate (GFR) by plasma clearance of 51 Cr-EDTA, renal blood flow (RBF) by clearance of 99 Tc MAG3 and performed kidney biopsy for light microscopy. Kidney volume (KV:) and resistive index (RI) on interlobar renal arteries were measured by Doppler sonography. In 11 pts we performed renal angiography using a score system (0-40) to analyse direct and indirect (parenchimal) signs of vascular lesions. Results: 16(39%) pts were allocated into CI, 13(31.7%) in CII and 12(29.3%) in CIII. Age and HbA1c were similar in the 3 groups while known D duration was shorter in CI than in CII and CIII (7.7±7.8 vs 14.3±8.5, 12.6±7.4 yrs, p<.05). Mean blood pressure was lower in N than in D (91±7 vs 105±8, 106±8, 104±7, mmHg, p<.005) and similar in the 3 groups of D. GFR was higher in CI than in CII (106±25 vs 87±24, ml/min/1.73m2, p<.05) and intermediate in CIII (94±37, ns.). RBF was higher in CI than in CII and CIII (518±62 vs 425±99, 409±145, ml/min/1.73m2, p<.05) with no differences between CII and CIII. All D had KV higher than N (311±79, 339±64, 295±42 vs 219±36, ml/1.73m2, p<.005); and among D group, CIII tended to have smaller kidneys (p=0.06). RBF/ml of KV was higher in CI than in CII and CIII (1.9±0.5 vs 1.5±0.4, 1.5±0.5, ml/min/ml, p<.05) without differences between CII and CIII. RI were higher in D than in N (RI: .7±.1, .7±.1, .7±.1 vs .6±.04, p<.005) and there was no difference between the 3 groups of D. Angiography score was greater in CII and CIII than in CI (10.5±2.1, 9.5±2 vs 5±2.3, p<.05) without differences between CII and CIII. RBF was inversely related to the angiography score (r=.83, p<.05). Conclusions: 1) Nephromegaly and increased RI are present in all D with AER+; among D, those in CIII tend to have smaller kidneys. 2) Lower RBF/ml of KV and higher angiography score, both parameters of macroangiopathy, are observed in D with typical diabetic glomerulopathy (CII) and atypical renal lesions (CIII), but not in patients in CI.

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INFLUENCE OF INSERTION/DELETION POLYMORPHISM IN THE ANGIOTENSIN CONVERTING ENZYME GENE ON THE PROGRESSION OF DIABETIC GLOMERULOPATHY IN IDDM PATIENTS WITH MICROALBUMINURIA.

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Aim: To investigate the influence of ACE-gene I/D polymorphism and its interaction with antihypertensive treatment (AHT) on the progression of diabetic glomerulopathy.

Methods: Thirty patients > 15 years old, with IDDM duration > 5 years and persistant microalbuminuria > 15 ug/min had renal biopsies taken at baseline and after 26-48 months of follow-up. Thirteen/30 patients (4 with II-genotype and 9 with ID+DD-genotypes) had been randomised to AHT (either enalapril or metoprolol) during study. ACE-genotype was determined by PCR. Glomerular structural changes were measured by stereological methods.

Results: Eight patients had II-, 19 ID-, and 3 patients DD-genotype. Basement membrane thickness (BMT), matrix star volume and overall diabetic glomerulopathy index (index DGP) were increased over study period in patients with ID+DD-genotypes only ($p<0.001$, $p=0.01$ and $p<0.001$ respectively). In patients with ID+DD-genotypes progression of BMT and index DGP were increased in those without as compared to those with antihypertensive treatment ($p<0.001$ and $p=0.02$ respectively.) In multivariate analysis ACE-genotype, AHT and mean HbA1c had an independent influence on progression of BMT ($p=0.02$, $p=0.01$ and $p<0.001$ respectively). When including an interaction term ACE-genotype by AHT, this variable and study mean HbA1c had significant influences on progression of BMT ($p<0.001$). The same was true when index DGP was the dependent variable ($p=0.01$).

Conclusion: Young IDDM patients with microalbuminuria carrying the D-allele have an increased progression of diabetic glomerulopathy. An interaction between this allele and abscens of antihypertensive treatment seems to be present. Our data need to be confirmed in large scale studies.

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ANGIOTENSIN I-CONVERTING ENZYME (ACE) GENE POLYMORPHISM AND RESPONSE TO ENZYME INHIBITION.

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Resently, an incertion deletion (I/D) polymorphism of the gene ACE was shown to account for 44% of the interindividual variability of plasma ACE levels. DD genotype is associated with higher plasma ACE levels and therefore a higher activity of the intrarenal renin - angiotensin system. We studied influence of the insertion/deletion (I/D) gene ACE polymor-phism on antiproteinuric effect of two ACE inhibitors - ENALAPRIL in dosage of 5 or 10 mg/24 and RAMIPRIL in dosage of 2,5 or 5 mg/24 - in young patients (ranged in age from 11 to 21, duration of the disease from 2 to 18 years) with diabetic nephropathy. 57 patients was with microalbuminuria and 11 ones with proteinuria. All the patients had normal blood pressure (BP) level. Creatinin and urea levels were normal. Glycolized hemo-globin HbA1 and albuminuria levels were determined by standard methods, I/D gene ACE polymorphism analysis was performed by isolating DNA from peri-pheral blood cells using polymerase chain reaction technique. The therapy was continued from 12 to 36 weeks depending on achievement of normal albuminuria level. Reliable difference between the antiproteinuric effect of the enalapril and ramipril was not observed. Hypotensive effect of therapy was developed only as tendency for decrease of the BP levels. Regression analysis had shown up: Antiproteinuric effect was reached in II genotype patients at minimum duration of the treatment and it depended on the rate of the compensation of the carbohydrate metabolism. More prolonged therapy was required for reaching the antiproteinuric effect in patients with ID genotype. The effectiveness of drugs decreased significantly in DD genotype patients independently on the duration of the treatment and the rate of the compensation of the diabetes. Thus, interference between the antiproteinuric effect of ACE inhibitors, the quality of metabolic control and the ID gene ACE polymorphism was observed.

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ANGIOTENSIN CONVERTING ENZYME GENE I/D POLYMORPHISM AND DIABETIC NEPHROPATHY IN IDDM CHILDREN AND ADOLESCENTS.

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Aims: to study association between I/D polymorphism of the angiotensin-converting enzyme (ACE) gene and diabetic nephropathy (DN) in children and adolescents with IDDM. Materials and methods: In order to enlarge the difference between DN «case» and DN «control» phenotypes the **DN«case»** group were formed of IDDM children and adolescents with early (less then 10 years after the diagnosis onset of DN (8,5÷1,4) (mean÷stdev)) and **DN«control»** group was formed of those, who had no signs of DN and diabetic retinopathy after 10 years of IDDM (11,8÷1,4). Groups didn't differ by age (15,7÷2,5) and sex. Results: the clinical characteristics of the groups and data of ACE genotyping are given in table 1.

	DN «case», n=42	**DN «control»**, n=30	p
Insulin dose, IU/kg	0,98÷0,26	0,88÷0,24	0,05
SBP/DBP, mmHg	122,2÷12,2/79,8÷10	112,8÷12,2/74,8÷6,6	0,002/0,01
Height SDS	-1,02÷1,5	-0,3÷1,3	0,02
Retinopathy	86%	0%	
Cataract	23,3%	0%	0,0004
Limited Joint Mobility	37,2%	20%	0,05
Delayed puberty and short stature	23,2%	6,6%	0,02
Diabetic neuropathy	97,7%	90%	0,1
Necrobiosis lipoidica	6,9%	0%	0,04
II	12,8%	41,6%	0,008
ID	61,5%	25%	0,003
DD	25,6%	33,3%	0,7
I/D	43,5%/56,4%	54,2%/45,8%	0,12
I/D	43,5%/56,4%	54,2%/45,8%	0,12

Conclusion: DN «case» group has more specific and nonspecific diabetic complications and higher HBA1. That confirm the major role of increased glycaemia on early development of complications of IDDM. But I/D genotype of ACE gene and its influence on RAS system activity is seemed to play some role in DN pathogenesis where II genotype contribute to protection from early DN development in children and adolescents.

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CONTRIBUTION OF ANGIOTENSIN I CONVERTING ENZYME I/D POLYMORPHISM TO THE DEVELOPMENT AND PROGRESSION OF NEPHROPATHY IN TYPE 1 DIABETES.

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Aim: To prospectively analyze the role of the I/D polymorphism of the angiotensin I converting enzyme (ACE) gene for the progression and development of diabetic nephropathy (DN). **Patients and methods**: From 1989 to 1996, we enrolled in a prospective folllow-up study 310 type 1 diabetic patients attending our clinic (age at disease onset< 40 years; diabetes duration>2 years; age 34 +/- 13 yr (m +/- SD) and sex ratio 0.57 (179 men ,131 women)). The following variables were recorded every 4-6 months until 1998, or death: physical examination, Systolic / Diastolic Blood Pressure (SBP / DBP), Urinary Albumin Excretion (UAE), HbA1c and plasma creatinine (Cr). The I/D polymorphism was determined by double PCR. The main outcome was the occurrence of a renal event defined as the progression from one stage of DN to a higher one: absent (normal UAE, Cr<150 µmol/l), incipient (microalbuminuria, Cr<150 µmol/l), established (proteinuria, Cr<150 µmol/l), advanced (Cr> or =150 µmol/l), terminal (renal replacement therapy). **Results**: At baseline, 251 patients (81 %) had no DN, 35 (11%) had incipient, 18 (6%) established, and 6 (2%) advanced nephropathy. The I/D polymorphism of the ACE gene was in Hardy-Weinberg equilibrium (54 *II*, 149 *ID*, 106 *DD*, $\chi2=0.02$; $p=0.89$) and not related to the stage of DN ($\chi2=5.51$; $p=0.48$). Median duration of follow-up was 6 years (range: 2-9). During follow-up, 2 *II*, 1 *ID* and 5 *DD* patients died ($\chi2=4.34$; $p=0.11$). Two *II* patients progressed in nephropathy compared to 32 *ID* and 16 *DD* patients (log-rank *II* vs *ID* vs *DD* = 8.57; $p=0.014$), with a protective effect of the *II* genotype (log-rank *II* vs *ID* or *DD* = 6.95; $p=0.0085$). To study if ACE I/D polymorphism affects the development of DN, we restricted the analysis to those patients having no nephropathy at baseline. The effect of the ACE genotype remained significant (log-rank *II* vs *ID* vs *DD* = 6.30; $p<0.05$, and *II* vs *ID* or *DD* = 6.28; $p<0.02$). Using the Cox's proportional hazards model for the 310 patients, the *ID* or *DD* genotype compared with the *II* genotype (relative risk (RR): 11.47, 95% CI: 1.57-83.91; $p<0.02$), the increase in 1 mm Hg of baseline SBP (RR: 1.04; 95% CI: 1.01-1.07; $p<0.008$) and the increase in 1% mean HbA1c during follow-up (RR: 1.32; 95% CI: 1.05-1.67; $p<0.02$) were independent risk factors for renal event, while gender, baseline UAE, diabetes duration, DBP or age at diabetes onset were not. **Conclusion**: The *II* genotype of the ACE gene is independently associated with a low risk of development and progression of DN in type 1 diabetes.

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Time-Dependent Medullary Thick Ascending Limb-Specific Na,K,ATPase Activty In Streptozotocin-Induced Diabetes Mellitus In Rat

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In rat, the medullary thick ascending limb (MTAL) Na,K,ATPase activity increases early after induction of diabetes by streptozotocin. This increase in Na,K,ATPases activity is in contrast with the concomitent decrease observed in the nerve, the red blood cell, and the cardiac myocyte. The MTAL represents a high fraction of the inner stripe of outer medulla. Long-term evolution of MTAL Na,K,ATPase activity during experimental diabetes is unknown. The **aim of our study** was to confirm the change of MTAL Na,K,ATPase activity after induction of diabetes, to observe the time-dependant evolution of Na,K,ATPase activity and to determine wheather this increase was dependent on a specific nephron segment. **Material and methods**: We studied the renal Na,K,ATPase activity respectively 6 (6W) and 12 weeks (12W) after induction of diabetes. Three groups were studied, one group served as control. Both kidneys were carefully dissected under binocular loop. Whole kidney tissue, whole medulla tissue, cortical tissue and the inner stripe of the outer medulla representing MTAL were isolated. Na,K,ATPase activity was measured by spectrophotometric determination of inorganic phosphate released from ATP, with or without ouabaïn. Results were expressed as nmolPi/mgprot/h. **Results**: Diabetes resulted in an increase of kidney weight after 6W and 12W (1,66±0,22 gr vs 2,01±0,2 gr $p<0,01$), as compared with controls (1,48±0,15 gr). Cortical tissue weight showed an increase between controls and 6W (0,74±0,25 vs 0,94±0,27 gr $p<0,01$) but no further increase at 12W (1,09±0,4 gr). There were no differences in Na,K,ATPase activity measured in the whole kidney tissue, the cortex or the whole medulla preparations in all groups. Conversely, diabetes resulted in an increase of the MTAL Na,K,ATPase activity 6W after the injection of streptozotocin (63287±21260 vs 31651±11430 $p<0,01$). At 12W, a decrease of Na,K,ATPase activity versus the controls was observed (7532±2470 vs 31651±11430 $p<0,01$). **Conclusion**: Our results confirm a diabetes-induced increase of MTAL Na,K,ATPase activity after 6W of diabetes. After 12W, a decrease in MTAL Na,K,ATPase activity is observed. This biphasic time-dependent evolution is likely to play a role in the adaptative mechanisms of kidney to diabetes. The renal Na,K,ATPase activity after induction of diabetes in rat is time-dependant and MTAL-specific.

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Nephropathy: Renal Function

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EFFECTIVE RENAL PLASMA FLOW AND FILTRATION FRACTION IN NON-DIABETIC AND NEWLY DIAGNOSED TYPE 2 DIABETIC SUBJECTS

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Aims: to determine 95% reference ranges for effective renal plasma flow (ERPF) and filtration fraction (FF) for non-diabetic subjects and to apply these to subjects with newly diagnosed type 2 diabetes. **Materials and Methods**: ERPF and glomerular filtration rate (GFR) were determined using ^{125}I-iodohippurate and ^{51}Cr-EDTA respectively and corrected for body surface area in 81 non-diabetic Caucasian subjects (41M, 40F) and 211 Caucasian subjects with newly diagnosed type 2 diabetes (150M, 61F). FF=GFR/ERPF (%). 95% reference ranges for ERPF and FF were calculated from non-diabetic subject data as mean ± k×SD where k is the appropriate tolerance factor for the sample size. Sex- and age-adjustments were investigated and the resulting 95% reference ranges applied to the diabetic subject data. **Results**: Non-diabetic subjects were aged 51 (30 to 74) years (median (range)) with fasting plasma glucose (FPG) 5.3 ± 0.5 mmol/l (mean ± SD), BMI 26.2 ± 4.8 kg/m^2, ERPF 545 ± 121 ml/min/(1.73m^2), GFR 102 ± 19 ml/min/(1.73m^2) and FF 19.1 ± 2.8%. Age- but not sex-adjustment, was necessary for ERPF, giving a reference range from 540–3.76×age to 1223–9.38×age ml/min/(1.73m^2). The reference range for FF was from 13.5 to 24.7%. Diabetic subjects were aged 54 ± 9 years with FPG 11.7 ± 3.2 mmol/l, BMI 29.5 ± 4.9 kg/m^2, ERPF 522 ± 118 ml/min/(1.73m^2), GFR 117 ± 22 ml/min/(1.73m^2) and FF 22.8 ± 3.1%. Applying the reference ranges, 8/211 (4% 95%CI[2-8%]) diabetic subjects had elevated (↑) and 10/211 (5%[2-9%]) depressed (↓) ERPF. 53/211 (25%[20-32%]) subjects had ↑FF. Of these, using previously calculated GFR reference ranges, 13/53 (25%[14-39%]) had ↑GFR and within range (↔) ERPF, 33/53 (62%[48-75%]) had ↔GFR and ERPF while 7/53 (13%[6-26%]) had ↔GFR and ↓ERPF. **Conclusions**: Age-adjustment is necessary for reference ranges for ERPF but not for FF. Sex-adjustment is not necessary for either. ↑diabetic FF is due to ↑GFR in 25% of cases and to ↓ERPF in 13% of cases with ↑FF.

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UNEXPLAINED DECREASE IN GLOMERULAR FILTRATION RATE AFTER REPEATED MEASUREMENTS IN TYPE 1 DIABETIC PATIENTS

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Constant infusion sinistrin (inulin) clearance with urine collection has been accepted as the golden standard in measuring glomerular filtration rate (GFR). To avoid urine collections single shot plasma sinistrin clearance methods have been devised. **AIM** of our study was to test the reproducibility of repeated single shot sinistrin clearance measurements in type 1 diabetic subjects and healthy controls. **METHOD:** Two GFR measurements (GFR1, GFR2) were done immediately consecutive. For determination of GFR we used a new single injection sinistrin clearance method which showed good correlation with classical constant infusion techniques with urine sampling. The calculation was performed by a two compartment modelling and the analysis of sinistrin showed no interference with glucose up to 1000mg/dl. **RESULTS:** 17 type 1 diabetic subjects (mean and SD), age 41 (10.7), systolic blood pressure (BP) 122 (12.0) mmHg, diastolic BP 78 (6.1) mmHg, diabetes duration 18 (10.1) years, HbA1c 8.6 (1.0)%, albuminuria 23.4 (31.7) mg/24h and 6 healthy controls, age 26 (2.6), systolic BP 117 (75.3), diastolic BP 75 (6.1) were examined.

	GFR1 (Mean and SD)	GFR2 (Mean and SD)	Δ GFR1-GFR2	p-value
Diabetic patients	130.9 (16.0)	118.3 (20.5)	-12.6	0.000
Control subjects	111.7 (11.4)	112.2 (12.2)	+0.5	0.319

In controls we found an excellent repeatability between GFR1 and GFR2 (Altman and Bland, mean of difference: 1 (SD2) ml/min/1.73m^2), however there was a highly significant and clinically relevant decrease in the second GFR reading in diabetic patients. This change could not be explained by protein intake, albuminuria, HbA1c, glycaemia or blood pressure. **CONCLUSION:** In contrast to normal subjects, in type 1 diabetic patients single injection sinistrin clearance technique showed a decrease in GFR on repeated measurements. Overestimation of GFR by the first sinistrin clearance may be possibly caused by an incomplete distribution of sinistrin in slow compartments, such as extracellular fluid compartment, muscle or fat tissue in diabetic patients.

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VARIABILITY OF ALBUMIN EXCRETION RATE IN PATIENTS WITH TYPE 2 DIABETES

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The presence of microalbuminuria indicates not only early renal damage but also an increased risk of cardiovascular disease. **Aims**: The aims of the study were to assess the within patient variability of overnight Albumin Excretion Rate (AER) in patients with type 2 diabetes and persistent microalbuminuria (MA). **Patients and Methods:** 20 patients with type 2 diabetes and MA (AER>20μg/min) were reviewed on 2 occasions one month apart. At each visit 3 consecutive overnight AERs were provided. **Results**: Median (range) age of patients was 61.2 [43-73] years, duration of diabetes 4.2 [1-12] years and HbA1c 8.0[6.5-11.0]%. The median (range) BP was 147 (110-190)mmHg systolic and 85 (78-108)mmHg diastolic with AER of 42 (22-133) μg/min. There was good reproducibility of AER over the short time period with mean +/- SD AER between the two visits of 58.6 +/- 18μg/min vs 56.2 +/- 21μg/min ($r=0.95; p<0.001$). A significant correlation between AER and diastolic ($r=0.89; p<0.001$) but not systolic ($r=0.16$;NS) BP or HbA1c ($r=0.18$;NS) was obtained. **Conclusions**: These results confirm that within patient variability of AER in patients with type 2 diabetes and persistent MA was negligible and that a single AER measurement was reliable and reproducible. Between patient variability, however, was high, ranging from 22 - 133μg/min. This correlated only with diastolic but not systolic BP and may have reflected the variable systolic but not diastolic readings obtained.

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BRAIN NATRIURETIC PEPTIDE INDUCES MICROALBUMINURIA IN TYPE 1 DIABETES.

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Background Atrial natriuretic peptide (ANP) increases the urine albumin excretion rate in type 1 diabetic (DM) subjects. Brain natroiuretic peptide (BNP) is structurally and functionally related to ANP, but it is not known if BNP also alters UAER. Aims To ascertain if intravenous infusion of BNP alters the UAER in subjects with type 1 DM and normal UAER. Materials and Methods We present the results of a randomised, double blind, placebo controlled study of the effects of BNP on the UAER. Eight type 1 diabetic subjects with normal UAER were studied on three occasions. Subjects were euglycaemic clamped and subsequently water loaded to steady state diuresis (20 mls kg^{-1} orally plus urinary losses). When in steady state, a one hour intravenous infusion of; placebo, ANP 0.025 μg $kg^{-1}minute^{-1}$, or BNP 0.025 μg kg^{-1} $minute^{-1}$ was administered. Urine was collected at 15 minute intervals for estimation of albumin creatinine ratio (ACR). Results were analysed by ANOVA. Results ACR was unaltered by placebo (1.3 ± 0.5 to 1.2 ± 0.4 mg $mmol^{-1}$, mean and SD, $p > 0.9$), but increased compared to placebo with infusion of both ANP (1.2 ± 0.4 to 13.4 ± 8.6 mg $mmol^{-1}$, $p = 0.0004$), and BNP (1.1 ± 0.4 to 9.8 ± 8.4 mg $mmol^{-1}$, $p = 0.0001$). Conclusion Intravenous infusion of BNP and ANP increase the UAER in type 1 diabetic subjects with normal UAER.

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DOPAMINE-INDUCED AER INCREASE MIGHT BE A PREDICTOR OF MICROALBUMINURIA IN TYPE 1 DIABETIC PATIENTS: A TEN-YEAR FOLLOW-UP STUDY.

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Aims: To evaluate the predictive power of the dopamine test for microalbuminuria in type 1 diabetic patients. **Materials and Methods:** A low-dose (2.5 μg/kg/min) i.v. dopamine (DA) infusion (30 minutes) has been administered to 62 type 1 normotensive, normoalbuminuric diabetic patients (28M, 34F; age 27±10 years, duration of diabetes 9±5 years) and 22 healthy controls. During DA, albumin excretion rate (AER) increased both in controls (from 3.74±1.0 to 21.7±2.81 μg/min) and in diabetics (from 6.09±2.81 to 31.3±34.9 μg/min). Prevalence of "responders" (19 out of 62; 31%), arbitrarily defined as those subjects who showed a DA-induced AER increase within the higher quartile (>40 μg/min) increased with diabetes duration: 2/17 (12%), 6/17 (35%) and 11/28 (39%) in subjects with <5, 6-10 and >10 years of disease, respectively. **Results:** Follow-up (11±1.8 years) was longer than 5 years (range 5-14) in 53 patients (duration of diabetes at follow-up 23±8 years). Microalbuminuria developed in 10 patients (19%), 5 out of 39 "non-responders" to dopamine (13%) and 5 out of 14 "responders" (36%) ($X^2=3.53$, $p=0.06$). Hypertension (>140/90 mmHg) developed in 7 patients (13%), 4 "non-responders" (10%) and 3 "responders" (21%) (ns). Retinopathy was present at baseline in 44% of subjects; after exclusion of the six patients with proliferative retinopathy at baseline who cannot progress, progression of retinopathy was no different between the two groups. **Conclusions:** The increased transglomerular leakage of albumin acutely induced by low-dose dopamine infusion might predict the development of microalbuminuria in normotensive normoalbuminuric subjects with type 1 diabetes.

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LIMITATIONS OF THE MICROALBUMIN/CREATININE RATIO IN DETECTING EARLY MICROALBUMINURIA IN TYPE 1 DIABETES

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The ratio of urinary microalbumin to creatinine (MCR) on a spot clinic sample of urine in Type 1 diabetic patients is an accepted screening test for microalbuminuria. Raised MCR > 2.5 - 3.5 have been used as thresholds for treatment of normotensive patients with ACE inhibitors, but greater predictive value might be obtained by establishing a rate of progression of MCR of over 5% per year (progression to proteinuria in 30 years). **Aim**: To determine the degree to which thresholds derived from group data may need re-evaluation in the light of within-subject variation in clinical tests when applied to individual patients. **Methods**: We have assessed the within-subject variation of microalbumin concentrations (MAC) and MCR in a 104 type 1 diabetic patients (64 M:40 F, median (range) age 39 (19 - 74) years, duration 21 (1-50) years), attending a routine hospital diabetic clinic from duplicate samples taken 6-12 months apart. **Results**: No significant rise in MCR was evident over this period. Logarithmic transformation of MAC and MCR produced uniform within-duplicate variance across the respective ranges of values 1 - 267 $mg.l^{-1}$ and 0 - 46 $mg.mmol^{-1}$, allowing calculation of an unbiased common within-subject SDs (SDw). SDw for log MCR (0.32) was 24% lower than that for log MAC (0.42), but represents a 1 SD range for a single determination of MCR of 48 - 210%, irrespective of the value of MCR. In an individual patient, to have 85% confidence that the underlying MCR exceeds 3 $mg.mmol^{-1}$, the MCR on a single sample should exceed 6.3 $mg.mmol^{-1}$ or the mean of two consecutive samples should exceed 5.1 $mg.mmol^{-1}$. To have 85% confidence that the underlying progression was greater than an annual increase of over 5% per year, the increase in an individual, if calculated by regression from 6 monthly values over 3 years, should exceed 45%. **Conclusion**: Assessment of the degree of microalbuminuria in an individual patient should take account of quantitative evaluation of the within-subject variation. This may significantly affect individual thresholds for clinical intervention.

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PRACTICAL ASPECTS OF SCREENING FOR EARLY RENAL IMPAIRMENT IN DIABETIC PATIENTS.

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Aims: Controversy still exists regarding the type of urine specimen to be used for detecting early renal impairment of diabetic patients. To assess reliability, albumin concentration (AC), albumin to creatinine ratio (A/C ratio) of first void urine sample and 24-hour urinary albumin excretion (UAE) in timed specimens were measured and compared. **Materials and Methods:** AC, A/C ratio and UAE were determined by immunoturbidimetric method 3 times within 3 weeks in 192 adult diabetic patients with suspected early renal impairment (men/women 136/56; IDDM/NIDDM: 90/102; age: 51.4±10.8 years; duration of diabetes: 15.3±9.1 years; serum creatinine: 94±20 µmol/l; BMI: 27.9±4.6 kg/m^2; HbA_{1c}: 8.5±1.5 %; actual blood pressure: 138±14/83±9 mmHg; x±SD). **Results:** According to the UAE values one third of patients (31.2%–30.7%–34.4%) were normoalbuminuric (<30 mg/24 hours), more than half of the patients (55.8%–57.3%–53.6%) proved to be microalbuminuric (30-300 mg/24 hours) while the remaining group of patients (13.0%–12.0%–12.0%) were macroalbuminuric (>300 mg/24 hours) at the three measurements, respectively. The probability of the results of the consecutive measurement being in the same range of albuminuria as the previous one was 80.4 % with UAE values, 73.3 % with A/C ratio and 76.6 % with the AC determination. Using log-transformed data the strongest correlation was found between A/C ratios and AC values in all series (r=0.92 – 0.88 – 0.86; p<0.001). **Conclusion:** Beside the standard method of UAE measurement in timed urine samples, the use of the more convenient urinary spot collection for AC and A/C ratio determination could also provide reliable results for detecting early renal impairment of diabetic patients.

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INSULIN SENSITVITY IN TYPE 1 DIABETICS WITH MILD NEPHROPATHY IS NOT ASSOCIATED WITH THE DEGREE OF ALBUMINURIA

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Aims and methods: Microalbuminuria can be a part of the insulin resistance syndrome and in addition diabetic nephropathy may cause insulinresistance. To elucidate insulin sensitivity and clearance in type 1 diabetics with mild nephropathy we utilized the euglycemic hyperinsulinemic clamp technique (insulin infusion at 56 mU/m^2/min) to study nine male and seven female patients, age 42.9±2.7 (mean± SEM) years, diabetes duration 30.2± 2.7 years, HbA1c 7.1±0.3 % and BMI 24.5±0.6 kg/m^2. Albumin excretion rate (AER) was measured in two overnight urine collections and glomerular filtration rate (GFR) was determined by ^{51}Cr EDTA clearance. Results: AER was 149± 41 µg/min (range 24-448) and GFR 105±4.2 ml/min/1.73m^2 (range 81-135) considered normal. Ten patients had micro- (20-200 µg/min) and six macroalbuminuria (>200 µg/min). Insulin sensitivity (M-value) was 7.2±0.7 mg/kg/min, insulin sensitivity index (ISI=100 x M/plasma insulin) 8.4±0.8 and insulin clearance (MCR-I) 17.7±1.0 ml/kg/min. Regression analyses showed no association between AER and M (r=0.03, p=0.91), ISI (r=0.03, p=0.90) or MCR-I (r=0.03, p=0.93). AER was associated with HbA1c (r=0.60, p=0.01) and serum triglycerides (r=0.48, p=0.06), both indicators of metabolic control. Insulin sensitivity was correlated to diabetesduration (r=0.50, p=0.05) but not BMI (r=-0.35, p=0.17), probably because all patients were non-obese. Conclusion: In type 1 diabetics with mild nephropathy (elevated AER but normal GFR) the degree of albuminuria does not seem to be directly linked to the degree of insulin resistance but as previously descibed to glycemic control.

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Nephropathy: Mechanisms

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GLYCOSAMINOGLYCANS PREVENT THE ENHANCED TGF-ß1 GENE EXPRESSION BY GLUCOSE IN MESANGIAL CELLS

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Aims: In diabetic rats therapy with glycosaminoglycans (GAGs) ameliorate development of glomerulosclerosis and albuminuria. Because GAGs prevent also the enhanced expression of TGF-ß1 by high glucose in cultured mesangial cells the aim of our study is to clarify the molecular mechanism of the reduced TGF-ß1 synthesis.

Materials and Methods: Porcine mesangial cells were transiently transfected with a plasmid containing the human TGF-ß1 promoter fragment -453/+11 fused to the luciferase gene to determine the effect of GAGs on the TGF-ß1 promoter activity in normo- and hyperglycemic conditions. Electrophoretic mobility shift assays (EMSA) were performed to study the different binding of nuclear proteins to AP-1 sites in the TGF-ß1 promoter after treatment with GAGs. The effect of the GAGs on the translocation of different PKC isoforms as possible mediators of the glucose-mediated stimulation were shown in western blots.

Results: The presence of GAGs prevented the glucose- and PMA-stimulated activation of the TGF-ß1 promoter dose- and time-dependently, 10 µg/ml medium were sufficient to abolish the up-regulation completely. The same concentration of GAGs inhibited the enhanced binding of nuclear proteins by PMA to AP-1 binding sites of the TGF-ß1 promoter, which mediates the glucose and PMA-effect. Binding of the ubiquitous transcription factor Sp1 to a consensus sequence, however, is not influenced by GAGs. 10 µg GAGs /ml medium prevented also the PMA-stimulated translocation of the Ca2+-dependent PKC isoforms from cytosol to the membrane fraction of mesangial cells.

Conclusions: The results revealed a down-regulation of the glucose-enhanced TGF-ß1 gene expression by GAGs through a reduced promoter activity. This reduction is possible mediated by a PKC-dependent mechanism, which leads to inhibition of AP-1 DNA binding activity.

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LATENCY-ASSOCIATED PEPTIDE INHIBITS TGF-β ACTIVITY IN HUMAN MESANGIAL CELLS

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【**Aims**】 TGF-β 1 has been thought as a key factor of the matrix accumulation in diabetic nephropathy. We investigate whether latency-associated peptide(LAP) inhibits TGF-β 1 activity and suppresses the glucose-induced increase in the synthesis of extracellular matrix proteins in cultured human mesangial cells(HMCs).

【**Materials and Methods**】 HMCs were cultured with either 5 or 33 mmol/l glucose in the absense or presense of the recombinant LAP. TGF-β 1, fibronectin(Fn), thrombospondin(TSP) produced by HMCs were measured by ELISA. Active TGF-β 1 was evaluated by the binding ability to soluble type Ⅱ receptor.

【**Results**】 LAP inhibited the binding of TGF-β 1 to soluble type Ⅱ receptor in vitro system. Effective dosages of LAP for 50% inhibition of TGF-β 1 activity was approximately 10-fold molar exess. The addition of LAP to HMCs resulted in the dose-dependent decrease in active TGF-β 1 secreted from HMCs. TGF-β 1-induced increase in Fn production from HMCs was also prevented by LAP in a dose-dependent manner. Exposure of HMCs to 33mmol/l glucose resulted in an increase in both Fn and TSP production compared with 5 mmol/l glucose. The glucose-induced increase in the production of Fn and TSP was prevented by the concomitant incubation with LAP, whereas no significant changes were noted when LAP was added to HMCs cultured with 5 mmol/l.

【**Conclusions**】 LAP suppresses the glucose-induced overproduction of extracellular matrix proteins by inhibiting active TGF-β 1 in HMCs. These findings warrant further in vivo study on the effect of LAP in diabetic nephropathy.

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ENHANCED GLUCOSE TRANSPORT INDUCED BY HIGH GLUCOSE AND TGF-β IN GLOMERULOSCLEROSIS- VS. HYPERTENSION-PRONE RATS

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Increased glucose transport in response to high glucose and hyperglycemia-induced TGF-β overexpression, possibly via upregulation of Glut-1, may participate in the accumulation of mesangial extracellular matrix (ECM) occurring in diabetes. We previously showed that high glucose-induced ECM upregulation is more pronounced in mesangial cells (MC) from rats of the Milan normotensive strain (MNS), glomerulosclerosis(GS)-prone, than in MC from rats of the Milan hypertensive strain (MHS), which do not develop renal disease. **Aim.** To assess whether MC from MNS rats of 1 and 8 months of age (i.e. before and after GS onset) show increased activation of glucose transport system, as compared with MC from age-matched MHS rats, thus contributing to the abnormal glucose-induced ECM overproduction. **Materials and Methods.** MNS and MHS MC were grown for 3-5 days under high glucose (HG, 30mM) vs. normal glucose (NG, 5.5mM) levels. At confluence, MC were exposed to either TGF-β (50pM) or vehicle. After 24h, facilitative glucose transport was measured as ^{3}H-deoxyglucose (^{3}H-DG) incorporation into monolayers over 30 min. **Results.** When grown in HG vs. NG, MC from 8-month old rats showed enhanced in ^{3}H-DG uptake, with increases which were significantly more marked in MNS than in MHS MC (+31% vs. +14%, p<0.05). TGF-β also stimulated ^{3}H-DG incorporation in both MC lines, with more pronounced increases in MNS than in MHS MC (+71% vs. +45% in NG and +104% vs. +54% in HG, p<0.001). Similar results were obtained in MC from 1-month old rats. **Conclusions.** These results indicate that predisposition to GS in MNS rats is associated with abnormal MC glucose transport activity in response to HG or TGF-β, which is already detectable before disease onset. This mechanism, possibly genetically determined, may amplify the effects of HG and associated TGF-β upregulation on ECM overproduction, thus leading to excess matrix deposition under conditions of hyperglycemia.

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THE ROLE OF OXIDATIVE STRESS INDUCED NF-kB IN THE DEVELOPMENT OF DIABETIC NEPHROPATHY

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Aims: To reveal the role of oxidative stress in the development of diabetic nephropathy, the transcription factors of glomeruli in diabetic rats were examined and the effect of antioxidant was tested.

Materials and Methods: We mesured the activities of redox-sensitive transcription factors, nuclear factor-kB (NF-kB) and activating protein-1(AP-1) with electrophoretic mobility shift assay in the control rats and streptzotosin-induced diabetic rats which were treated or not treated with N-acetylcysteine. The changes in the transforming growth factor β (TGF-β) m-RNA contents and the urinary albumin excreation (UAE) were also examined. **Results:** Increased activity of NF-kB, but not AP-1 was found in the glomeruli of 4 and 12 weeks diabetic rats (p<0.05). The m-RNA content of TGF-β was increased in the glomeruli of 4 and 12 weeks diabetic rats, and their UAE was increased in 12 weeks diabetic rats (p<0.005). The oral administration of N-acetylcysteine prevented the elevation of activity of NF-kB without any change in the plasma glucose concentration (P<0.01), the m-RNA content of TGF-β and the UAE (Control 1.3±0.3 mg/day, DM 3.5± 0.7, DM+NAC 3.9±0.3) were not improved.

Conclusion: These results suggest that elevated oxidative stress is involved in the activation of the NF-kB in glomeruli of diabetic rats, but may not have a role in the development of nephropathy.

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VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION IN MICRODISSECTED GLOMERULI OF TYPE 2 DIABETIC PATIENTS.

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VEGF is mitogenic for endothelial cell and a potent enhancer of albumin permeability. In normal human kidney VEGF is expressed by glomerular podocytes and its binding to glomerular endothelial cells might be a paracrine regulatory mechanism of glomerular filtration barrier. It is expressed in 3 splice variants, characterized by 121, 165 and 189 aa, that differ in their diffusibility (121>165>189), in their mitogenic effect (165>121) and, possibly, in their action on glomerular permeability. It is unknown whether VEGF could contribute to determine diabetic glomerulopathy. **Aims**. Since to date there is no quantitatively and qualitatively informations on VEGF expression in glomeruli of diabetic patients, our aim was to evaluate VEGF mRNA expression in microdissected glomeruli obtained from kidney biopsies of diabetic patients. **Material and methods.** We studied 12 type 2 diabetic (D2) patients. Data are expressed as mean±SD. Age was 54.2±4 years; D2 duration was 9±7.1 years; HbA1c was 9.18±1.3%; 7 patients had microalbuminuria (20-200 µg/min) and 5 proteinuria (>200 µg/min). Biopsies were analysed by light, electron and immunofluorescence microscopy and divided in 2 groups: D2 with (5 pts) and without (7 pts) typical diabetic glomerulopathy. 4 to 15 glomeruli were microdissected from 1 mm^3 of cortical tissue of each renal specimen; VEGF mRNA expression was studied by RT/PCR comparative kinetic approach using, for quantitative analysis, the G3PDH gene as internal standard. We used 2 different sets of primers, spanning or not the splice sites of VEGF gene, in order to identify the 3 different isoforms and to quantify VEGF mRNA at glomerular level. **Results**. We found no differences in VEGF mRNA levels between patients with and without diabetic glomerulopathy (VEGF/G3PDH: 0.74±0.39 vs 0.90±0.67, n.s.); the predominant isoform in all patients was the smaller and more diffusible one (VEGF121); the 121/165 isoforms ratio was significantly higher in glomeruli characterised by typical diabetic leasions (6.7±2.8 vs 2.5±1.6, p<0.049). **Conclusions**. Our data suggest that typical diabetic glomerulopathy is not associated to a quantitative alteration of VEGF mRNA expression, but is related to a greater expression of the most diffusible VEGF isoform. This might lead to resident glomerular cells abnormalities.

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PLASMA sTNFR1 LEVELS AND INCIPIENT DIABETIC NEPHROPATHY IN TYPE 2 DIABETES MELLITUS

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Aims: Different cytokines have found to be involved in the development of experimental diabetic nephropathy. Circulating levels of the soluble fraction of tumor necrosis factor receptors (sTNFRs) are thought to reflect prevailing TNF-α action. We aimed to study plasma sTNFRs in relation to histological kidney changes in type 2 diabetes mellitus. **Materials and Methods:** Twenty-two patients (16 men) were prospectively studied [mean age 56 years (range 32-64), mean diabetes duration 13 years (1-29), body mass index 26.9 kg/m^2 (23.1-32.9), HbA1c 7.7% (5.4-12.6)]. Functional and renal optic histomorphometric studies were performed in all patients.

Results: The results disclosed a mean isotopical glomerular filtration rate of 126 cc/min (85-177), urinary albumin excretion of 39 mg/day (14-154), mesangial expansion 0,20 % (0.10-0.30) and interstitial fraction of 16,6 %. (6,5-32.5). Plasma sTNFR1 concentration correlated with mesangial expansion (r=0.59, p=0.004) and with interstitial fraction (r=0.58, p=0.005). After controlling for age, only the correlation with mesangial expansion persisted significant (r=0.46, p=0.038). Plasma sTNFR2 levels were not associated with histological changes. **Conclusions:** the results of the present study suggest that TNF-α might be involved in the progression of diabetic nephropathy.

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PROTEASE ACTIVITY FOR URINARY INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-3 IS INCREASED IN DIABETIC NEPHROPATHY

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The insulin-like growth factor (IGF) system has been implicated in the development of animal models of diabetic nephropathy. IGF binding protein-3 (IGFBP-3) modulates IGF actions and proteolysis of this IGFBP decreases its binding affinity for IGFs. **Aim**: This study aimed to investigate the protease activity for IGFBP-3 in urine from diabetic patients with micro- (albumin excretion rate:20-200 μg/min) and macroalbuminuria (>200 μg/min). **Materials and Methods:** We examined urine in patients with type II diabetes (n=33, age:55.8$\pm$8.7 years, 16women/17men) and in healthy volunteers (n=9, age:37.6 $\pm$14.5 years, 4women/5men). Aliquots of 24 hour urine collections were dialyzed and lyophilized. IGFBP-3 was analyzed by Western Immunoblotting (WIB). Proteolysis assay was performed using iodinated recombinant human IGFBP-3 as a substrate and measuring the percentage disappearance of intact tracer. **Results:** WIB showed IGFBP-3 bands sized 37-46 kDa, 30 kDa and 14-21 kDa in control urines and in urine from normoalbuminuric diabetic patients. The 14-21 kDa band was a proteolytic fragment. The urine of diabetic patients with micro- and macroalbuminuria contained less intact IGFBP-3, whereas levels of fragmented IGFBP-3 were increased. The urinary protease activity in micro- (n=12) and macroalbuminuric (n=12) groups (77$\pm$26% and 84$\pm$24%) was significantly higher than those (29$\pm$9% and 49$\pm$15%) in the normoalbuminuric (n=9) and the control groups (p=0.0001). The protease activity was inhibited by several protease inhibitors. **Conclusion:** Diabetic nephropathy is associated with proteolysis of urinary IGFBP-3. Since similar changes were not observed in patients' sera, this is likely to reflect changes in the kidney or urinary tract and may result in increased bioavailability of IGFs in the kidney.

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TRANSMEMBRANE ELECTRON TRANSFER IN DIABETIC NEPHROPATHY.

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Erythrocytes (RBC) reduce extracellular ferricyanide to ferrocyanide by transmembrane transfer of reducing equivalents involving ascorbate recycling. A cellular redox balance should link membrane thiol and plasma thiol. Since ascorbate regeneration is largely glutathione (GSH) dependent and cells have been suggested to be GSH depleted in diabetes mellitus, we measured RBC GSH, plasma sulfhydryl groups (SH groups) and ferricyanide reduction in 30 type 1 diabetic patients (age 34±10 y, disease duration 20±8 y; 10 without diabetic complications, 10 with retinopathy, 10 with nephropathy) and 30 matched healthy volunteers (36±10 y). Fasting plasma glucose was 15±7 mmol/l (vs 5±1 in controls, p<0.001), HbA1c 8.4±1.5% (vs 5.4±0.3, p<0.001), RBC GSH 0.76±0.12 mg/ml packed RBC (vs 0.88±0.18, p<0.01), SH groups 401±72 nmol/l (vs 444±56, p<0.05), ferrocyanide generation 15±5 µmol/ml RBC h (vs 13±5, ns). In comparison with 10 matched normoalbuminuric diabetics, ten patients with diabetic nephropathy had similar fasting plasma glucose, HbA1c, and SH groups, yet lower RBC GSH (0.73±0.08 vs 0.85±0.11, p<0.05) and higher ferrocyanide generation (18±4 vs 14±5, p<0.05). The rate of transmembrane transfer of reducing equivalents was associated with the urinary albumin excretion rate (UAER, R 0.3, p<0.05). We conclude that erythrocyte glutathione content as well as plasma thiol are reduced in albuminuric type 1 diabetic patients, who also show the highest rate of transmembrane electron transfer. We suggest that depletion of cellular GSH could reflect the extent to which cells support higher reducing equivalent recycling. Cytosolic reductive stress, produced by impaired oxidation of NADH to NAD, increases production of superoxide. It is a candidate mechanism of early glomerular dysfunction, manifested as increase in blood flow and albuminuria. Erythrocyte transmembrane electron transfer may be a useful tool to directly monitor cytosolic redox state and, moreover to evaluate efficacy of preventive interventions.

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ECONOMIC EVALUATION OF ANGIOTENSIN CONVERTING ENZYME INHIBITORS IN THE PREVENTION OF DIABETIC NEPHROPATHY

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Introduction: 2-4% of the population are diabetic, accounting for 7% of national health spending. Diabetic nephropathy is a common long term complication associated with high morbidity and mortality. **Aims:** To assess the cost-effectiveness of the early use of ACEi treatment compared to no ACE treatment in Type 1 diabetic patients. The hypothesis is that the early use of ACEi will delay or prevent disease progression (diabetic nephropathy) to End-Stage Renal Disease (ESRD) and is cost saving. **Method:** A Markov model was created to simulate progression over 30 years and commences 5 years after diagnosis of IDDM. Transition probabilities and resource use were derived from the literature and clinical experts. **Results:** Patients treated with an ACEi remained in the normoalbuminuric health state for longer (19.1 Versus 13.41years respectively) indicating that disease progression for patients treated with an ACEi was slower than the comparator group. Treatment with ACEi's costs £414 more than the comparator over 30 years. The higher cost for initial ACEi treatment is counterbalanced by the cost of ESRD in the no ACEi group. One extra year of life gained using ACEi's would cost £164. ACEi treatment will increase average survival by 21% thus patients treated with ACEi's can expect to survive on average 2.54 years longer than the comparator group giving an additional 3.2 years of time free from dialysis, death or transplantation. The time spent on dialysis or post transplant for the ACEi treated group is 0.4 years compared with 1.1 years for the no ACEi group. Furthermore, 5 and 25 patients would need to be treated with ACE inhibitors to prevent one additional death and one additional case of ESRD over 30 years respectively **Conclusions:** The use of ACEi 5 years after diagnosis of IDDM and for a 30-year period is cost-effective compared to treatment without an ACE.

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THE EFFECT OF LOSARTAN ON URINARY ALBUMIN EXCRETION IN TYPE 2 DIABETIC PATIENTS

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Albuminuria has been becoming the early indicator of nephropathy in diabetic patients. ACE inhibitors have beneficial effects on the reduction of urinary albumin excretion (UAE). Losartan is a novel angiotensin II receptor antagonist, which is an advanced form of ACE inhibitors. **Aims:** We have investigated the effect of Losartan on UAE in type 2 diabetic patients. **Subjects and Methods:** We divided the subjects into 3 groups: 1) 13 subjects who received 50 - 100 mg daily of Losartan alone or with Ca^{++} antagonists (Amlodipine or Nifedipine), 2) 15 who received ACE inhibitors such as Captopril or Enalapril, and 3) 20 who received Ca^{++} antagonists for 15 weeks. Systolic blood pressure was controlled between 120 ~ 139 mmHg. **Results:** Eleven of 13 subjects who received Losartan has shown significant reduction of UAE from 60 $\pm$ 14 to 45 $\pm$ 13 mg/gCr ($p<0.05$) (mean $\pm$ SEM). Five of 13 decreased UAE less than 20 mg/gCr. Twelve of 15 subjects who received ACE inhibitor decreased UAE from 58 $\pm$ 11 to 45 $\pm$ 13 mg/gCr ($p<0.05$). However, subjects who received Ca^{++} antagonists showed no change in UAE from 58 $\pm$ 24 to 64 $\pm$ 19 mg/gCr. In comparison with Losartan and ACE inhibitor groups, there were no significant differences in the effects on reduction of UAE. **Conclusion:** These results suggest that Losartan, as similar to ACE inhibitor, could have a beneficial effect on the early stages of diabetic nephropathy in the prevention of overt diabetic nephropathy.

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LOSARTAN REDUCES ALBUMINURIA IN NORMOTENSIVE TYPE 1 AND TYPE 2 DIABETIC PATIENTS

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In order to examine the effect of an ACE inhibitor, enalapril, and an angiotensin II antagonist, losartan on albuminuria, urinary albumin excreation (UAE) were measured at baseline, after 6 weeks losartan 25 mg/day, a 30-day washout period and 6 weeks enalapril treatment respectively, in 20 normotensive patients (age 39.8 ± 13 years, 10 women and 10 men) with type 1 (n=11) and type 2 (n=9) diabetes mellitus with persistent albuminuria. At baseline and after the each treatment period the following measurements were performed: body weight, office blood pressure, plasma glucose, urea, creatinine, sodium, potassium, transaminases (AST, ALT), lipids (total cholesterol, HDL and LDL fractions, triglycerides), albuminuria (three 24 h. collections) and creatinine clearance. Both the treatments were well tolerated. The significant and similar declines in the means±SD levels of UAEs were observed after both enalapril and losartan treatment:

	UAE (mg/day)	p
Before vs. after enalapril	490±158 vs. 363±122	0.03
Before vs. after losartan	385±113 vs. 325±101	0.014
Enalapril vs. losartan	%25.9 decrease vs. %15.5 decrease	0.07

The body weight, blood pressure, plasma glucose, urea, creatinine, sodium, transaminases (AST, ALT) and lipids (total cholesterol, HDL and LDL fractions, triglycerides) were unchanged after the both treatment. The creatinine clearances were significantly reduced whereas serum levels of potassium were increased after both enalapril and losartan treatment. In conclusion losartan is effective as well as enalapril in the treatment of normotensive diabetic patients with persistent albuminuria.

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LONG-TERM RENOPROTECTIVE EFFECT OF NISOLDIPINE AND LISINOPRIL IN PATIENTS WITH DIABETIC NEPHROPATHY

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Aims: To compare the long-term effect on glomerular filtration rate (GFR) of a long-acting calcium antagonist (nisoldipine) versus a long-acting angiotensin converting enzyme inhibitor (lisinopril) in diabetic nephropathy. **Material and Methods:** We performed a 4-year, randomised, double-dummy, controlled study comparing nisoldipine (20-40 mg once daily) with lisinopril (10-20 mg once daily) - double-blinded for the first year and single-blinded hereafter. Diuretics were required in 14 patients in each group. Included was 51 hypertensive Type 1 diabetic patients with diabetic nephropathy. Three patients dropped out during the first month, and results for the remaining 48 patients are presented. Rate of decline in GFR (Cr-EDTA) and 24-h ambulatory blood pressure (TM2420, A&D) was determined every 6 months. Albuminuria (ELISA) from three 24-h samples was measured with 3 months interval. **Results:** At baseline, the two groups were comparable: GFR (mean(SE)) 85 (5) and 85 (6) ml/min/1.73m^2, mean 24-h blood pressure 108 (3) and 105 (2) mmHg, and albuminuria (geom.mean (95%CI)) 1554 (980-2465) and 1033 (760-1406) mg/24h in the lisinopril and nisoldipine group, respectively. The mean follow-up time was similar, 45 months in the lisinopril and 43 months in the nisoldipine group. Mean arterial blood pressure was similarly reduced in the two groups to 100 (2) and 103 (1) mmHg in the lisinopril and nisoldipine group, respectively (NS, comparing changes between groups) . The rate of decline in GFR during the 4 years did not differ between groups: 6.5 (1.1) and 5.5 (1.6) ml/min/year in the lisinopril and nisoldipine group, respectively (NS). Two patients in the lisinopril and three patients in the nisoldipine group entered therapy for end stage renal failure. Albuminuria was reduced (% reduction from baseline) 52 % (95%CI: 14 to 73) in the lisinopril group, whereas it increased 12 % (-10 to 40) in the nisoldipine group, $p < 0.001$. **Conclusions:** Long-term treatment with lisinopril and nisoldipine have similar beneficial effects on progression of diabetic nephropathy in hypertensive Type 1 diabetic patients.

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BENEFICIAL EFFECT OF NITECAPONE, AN ANTIOXIDANT AND INHIBITOR OF CATECHOL-*O*-METHYLTRANSFERASE ON RENAL DYSFUNCTION IN DIABETIC RATS

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The cause of diabetic complications is multifactorial. Oxidative stress is considered to be one of the most important pathophysiological factors. Here we have tested the effect of Nitecapone (N), a compound that is both an antioxidant and an inhibitor of the dopamine metabolizing enzyme catechol-*O*-methyl transferase (COMT), on renal dysfunction in rats with streptozotocin induced diabetes. Daily administration of N abolished hyperfiltration and increased sodium excretion via a dopamine dependent inhibition of tubular Na^+, K^+ ATPase activity. (Glomerular filtration rate in N treated [N^+] versus N untreated [N^-] rats: 2.5±0.1 vs. 3.3±0.3 ml/min, $p<0.04$; fractionated sodium excretion in N^+ vs. N^- rats: 0.4±0.15 vs. 0.22±0.08 %, $p<0.05$). The effect of N on albuminuria and on the development of glomerulosclerosis was studied in rats with genetic predisposition to salt sensitive hypertension, which are known to develop advanced diabetic nephropathy. N administration significantly attenuated increased albumin excretion rate (N^+ vs. N^- rats: 3,4, range 0.03-9.8 vs. 10.9, range.1-26.0 mg/100 ml GFR, $p<0.01$) and the incidence of focal glomerulosclerosis (4.2±0.8 vs. 7.3±1.1 %, $p<0.05$) As a sign of oxidative stress, gene expression of the enzyme superoxide dismutase (SOD) was significantly up-regulated in the diabetic rats. Treatment with N has completely normalized the increased gene expression. The beneficial effect of Nitecapone might be attributed both to antioxidant and COMT inhibiting activity of the compound.

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EFFECTS OF AMINOGUANIDINE ON GLOMERULAR BASEMENT MEMBRANE ANIONIC CONTENT: AN ELECTRON MICROSCOPIC STUDY

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Aminoguanidine (AG) has previously been shown to prevent glomerular basement membrane thickening and albuminuria in diabetic rats. The **aim** of this study was to investigate the effect of AG on: 1-glomerular basement membrane (GBM) thickness 2-heparan sulfate (HS) content which represents anionic charge and 3-urinary albumin and HS excretion, in Streptozotocin induced diabetic rats. **Materials and Methods:** After diabetes induction female wistar rats were divided into 2 groups. Group A (n=11) received 1 g/L aminoguanidine carbonate in drinking water, group B(n=12) was given only tap water. Control rats received AG (group C,n=8) or tap water (group D,n=8). At the end of an 8 week period 24-hour urine collections were made and kidneys dissected. Urinary albumin was measured by electrophoresis, glycosaminoglycan excretion was detected by 1,9 dimethylene blue based spectrophotometry. Glomerular basement membrane HS content was measured by alcian blue stained particle count by electron microscopy.

Results:	albuminuria	urinary HS	GBM thickness	alcian blue stained
	mg/d	mg/d	μ	particle count
Group A	162,5±21,8	1050,6±195***	114,6±4,1	90,6±0,6
Group B	277,4±37,5*	2075,6±505 *	224±20,*	36±2,4**
Group C	106±27	899,4±158	95,7±7,6	93,8±1,1
Group D	82,4±10,7	956±68,7	97,3±4,3	98,6±1,7

*p<0,05,**p<0,01 vs other groups,*** p<0,01 vs group B

Diabetic rats showed a lower count of alcian blue stained particles on GBM while AG treated diabetic rats had counts similar to healthy rats. **Conclusion:** Aminoguanidine prevents the decrease in GBM anionic charged molecules and GBM thickening. This can be one of the mechanisms by which AG decreases albuminuria in diabetic rats.

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THIAZOLIDINEDIONES PREVENT STREPTOZOTOCIN-INDUCED DIABETIC RATS FROM DEVELOPMENT OF NEPHROPATHY

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We have recently found that thiazolidinediones (TZD) suppress the cytokine-induced production of monocyte chemoattractant protein-1 from cultured mesangium, and assumed that TZD may ameliorate the progression of the diabetic nephropathy. Here, we tested this hypothesis in vivo. Spontaneous hypertensive rats (SHR) (8w) were rendered insulinopenic diabetes by intravenous injection of streptozotocin (STZ) (50mg/kg). Diabetic rats were divided into three groups as follows:
(1) STZ-SHR given normal chow (STZ), (2) STZ-SHR given chow mixed with 0.1% troglitazone (STZ+tro), (3) STZ-SHR given chow mixed with 0.001% pioglitazone (STZ+pio). Non-diabetic SHR were divided into three groups as well: (4) SHR, (5) SHR+tro, (6) SHR+pio. TZD affected neither blood pressure (BP) nor blood glucose levels (BG). Albumin excretion rate (AER) markedly increased in STZ (SHR, 0.55±0.03; STZ, 1.68±0.23mg/day/creatinine clearance (Ccr)). TZD treatment significantly decreased AER at 12 weeks of treatment (STZ+tro, 0.84±0.26; STZ+pio, 0.71±0.09) without affecting Ccr. Histologically, TZD significantly inhibited the STZ-induced mesangeal expansion and loss of anion sites on glomerular basement membrane. In conclusion, TZD decreased AER without affecting BP, BG and Ccr. These results lighten novel therapeutic aspect of TZD on diabetic nephropathy.

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REVERSAL OF GALACTOSE-INDUCED RENAL HYPERPERFUSION IN RATS BY ALDOSE REDUCTASE INHIBITOR ZOPOLRESTAT

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Aims: Chronic elevation of renal blood flow and capillary pressure may be an important pathogenic factor in the development of diabetic nephropathy. Although prevention of renal hyperperfusion in diabetic and galactosemic rats with aldose reductase inhibitors (ARIs) has been reported, the reversibility of pre-existing renal hyperperfusion with an ARI has not been shown before. **Materials and Methods:** Male Sprague-Dawley rats were given free access to diet containing either 30% dextrin (Group D; n=11) or 30% D-galactose (Group G; n=21) for 14 days, at which time 14 of the galactose-fed rats were treated for 6 days with ARI zopolrestat (Alond™) (Z) at ~25 mg/kg BW/day mixed into the galactose diet (Group Z; n=14). At day 20 each rat was anesthetized with Inactin, 100 mg/kg, i.p., the left kidney was exposed via laparotomy, and superficial renal cortical blood flow (SRBF) was measured via laser Doppler flowmetry (Perimed PF3, Sweden). **Results:** Rats fed the galactose-rich diet had a slightly decreased body weight [mean±SD (n)]: 285±15 g (7) vs. 304±17 g (11), Group G vs. Group D ($p<0.05$, t-test), which was unaffected by Z: 288±14 g (14), Group Z. The galactose diet caused a 23% increase in left kidney weight: 1.6±0.1 g (7) vs. 1.3±0.1 g (11), Group G vs. Group D ($p<0.05$), which was unaffected by Z: 1.6±0.1 g (14), Group Z. SRBF was elevated 9% in the galactose-fed vs. dextrin-fed group: 284±9 (7) perfusion units (PFU) vs. 261±18 (11) PFU, Group G vs. Group D ($p<0.05$). Galactose-induced hyperperfusion was completely reversed by Z: 260±23 (14) PFU, Group Z ($p<0.05$ vs. Group G; NS vs. Group D). A repeat study yielded very similar results. **Conclusions:** These results demonstrate for the first time in an experimental model of diabetes that treatment with an ARI (Z) can reverse pre-existing renal hyperperfusion, a possible etiological factor for diabetic nephropathy.

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Effects of Tolrestat and Aminoguanidine on Cataract Formation, Retinopathy and Nephropathy of Diabetic Rats

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Aims: Drugs which interfere with sorbitol pathway and non-enzymatic glycosylation are under intense research to prevent chronic degenerative complications of diabetes. In this study ocular and renal diabetic complications of rats were investigated in a 24 weeks trial with tolrestat or aminoguanidine.

Materials and Methods: We investigated ocular and renal histopathological changes of male, Sprague-Dawley rats made diabetic with streptozocin. Study groups were made up of 10 normal controls (NC), 10 diabetic controls (DC), 10 diabetic tolrestat (DT) (25 mg/kg/day) group, 10 diabetic aminoguanidine (DA) (1gr/L in drinking water) group. Weekly ocular examinations and histopathological findings of the lens, retina and the kidneys had been studied in all four of the groups after sacrification of the surviving rats by the 24th week of streptozocin diabetes.

Results: None of the surviving 10 rats developed cataracts, retinal or renal histopathological changes from NC group. Surviving 5 rats from DC group all developed cataracts where as only one of the surviving 6 rats from DT group developed cataracts ($p=0.015$). All 5 surviving rats from DA group also developed cataracts although at a slower rate than the DC rats. Retinal histopathological findings were indifferent in between the diabetic groups by the end of the 24th week. DC showed outstanding mesangial proliferation, diffuse and focal glomerular basal membrane (GBM) thickening where as DA group showed no mesangial changes and minimal diffuse GBM thickening. DT group had similar findings with DC group although mesangial changes were less prominent in this group.

Conclusions: Our results show that chronic degenerative complications of diabetes could be partially prevented or delayed by the inhibitors of sorbitol pathway in the short term and non-enzymatic glycosylation in rats but on the long-term modification of glucose by other pathways may cause similar complications.

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Nephropathy: Epidemiology and Other

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ETHNIC DIFFERENCES IN DIABETIC NEPHROPATHY - THE UKPDS

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Previous epidemiological studies have suggested that diabetic nephropathy occurs more commonly in South Asian and Afro-Caribbean individuals than in whites, but prospective data are sparse. **Aims**: To evaluate whether South Asian (SA) and Afro-Caribbean (AC) individuals with type 2 diabetes are more likely than whites to develop nephropathy. **Methods**: 4692 individuals (83% white, 10% SA and 7% AC) aged 25 -65 years in the United Kingdom newly diagnosed with diabetes and without albuminuria were included. Proportional hazards regression was used to evaluate the association between ethnicity and nephropathy defined as microalbuminuria (> 50 mg/L) or more severe renal dysfunction, ascertained by two consecutive annual measurements. Ethnicity was evaluated alone, as well as in a model (n=2599) adjusted for the potential confounders of age, sex, haemoglobin A1c, systolic blood pressure, waist hip ratio, smoking, triglycerides and retinopathy measured 3 months following diagnosis of diabetes. **Results**: 947 individuals developed nephropathy during a median 9.5 years of follow-up. In a univariate model, neither SA nor AC ethnicity was associated with increased risk. However, significant ethnic differences existed between risk factors for nephropathy; e.g., relative to whites, South Asians were younger at diagnosis of diabetes and had lower blood pressure. Adjusted for potential confounders, relative to whites, South Asian ethnicity was associated with an increased risk of nephropathy with a hazard ratio of 1.4 (95% confidence interval 1.1-1.9); no increased risk was observed for Afro-Caribbean individuals. **Conclusions**: South Asian ethnicity was independently associated with an increased risk for nephropathy suggesting that in this group, prevention and management of hyperglycaemia and hypertension is of particular importance.

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ETHNIC DIFFERENCES IN A DIABETIC RENAL CLINIC IN BIRMINGHAM, UK

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Aims: To examine whether there were differences in the characteristics of patients referred to a diabetic renal clinic based on their ethnicity. **Materials and Methods:** A retrospective analysis of all patients referred to a diabetic renal clinic over an eighteen month period. 151 patients with diabetes (102 male) were referred because of persistent proteinuria and/or a raised creatinine. **Results:** Mean age at referral was 61.2 ± 1.06 years (range 16.9-85.1) and duration of diabetes was 14.2 ± 0.7 years (range 1-40). 131 (87%) patients had type 2 diabetes. 69 (45.7%) patients were of Asian origin (A), 46 (30.5%) were White Caucasians (WC) and 36 (23.8%) were of Afro-Caribbean extraction (AC). These proportions are similar to those attending the general diabetes clinics at City Hospital (A 42.8%, WC 36.8%, AC 20.4%). Prevalence of persistent dipstick positive proteinuria in the main diabetes clinics (sample size 525 patients) did not differ in the three groups (8.0% (A), 8.8% (WC), 8.4% (AC). The Asian patients were younger than both the other groups (57.7 ± 1.7 (A) vs 63.6 ± 1.9 (WC) vs 64.5 ± 1.6 (AC) years; p=0.012 ANOVA), but did not differ in their known duration of diabetes. 33 patients had renal biopsies (19 (A), 9 (WC), 5 (AC)).A diagnosis of diabetic nephropathy was confirmed in 16 (10 (A), 2 (WC), 4 (AC)). The next most common diagnosis was IgA nephropathy (8 patients (6 (A), 2 (WC)). **Conclusions:** The vast majority of the patients had type 2 diabetes. Based on previous studies we were surprised that the prevalence of proteinuria was not higher in the Asian or Afro-Caribbean patients and that Asian patients were not over-represented in our renal clinic. There may of course be a bias in referrals of diabetic patients to this hospital-based diabetes clinic. Previous findings of a higher incidence of proteinuria and diabetic renal disease in Asian patients in Leicestershire (mainly of Gujurati origin) were not confirmed in this clinic where the Asian patients are mainly of north Indian, Pakistani and Bangladeshi origin.

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OUTCOME OF DIABETIC NEPHROPATHY IN TYPE 2 DIABETES: ETHNIC DIFFERENCES

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Aims: To study the natural history of diabetic nephropathy in type 2 diabetes mellitus with special reference to ethnic differences. **Patients and Methods:** Type 2 diabetic patients referred to a specialist diabetes renal clinic with proteinuria (≥0.5g) were entered into the study. All patients notes were analysed retrospectively. **Results:** 169 (100 Caucasians, 69 non-Caucasians) type 2 diabetic patients were entered into the study and were followed up for median (range) 4 (2-10) years. 39 (39%) Caucasians and 23 (33%) non-Caucasians died. Caucasians had lower geometric mean HbA1c at referral [9.9 (9.4-10.4) vs 10.8 (10.1-11.6)%; p=0.03], higher baseline proteinuria [2.1 (1.7-2.6 vs 1.7 (1.2-2.4) g/24hr; p=0.07] and baseline creatinine [145.8 (130.1-163.3) vs 123.2 (105.5-143.8) μmol/l; p=0.02]. Caucasian subjects who died had higher baseline creatinine [188.4 (152.7-233.4) vs 158.9 (102.8-245.7 μmol/l; p=0.05]; coronary artery disease (CAD) [61 vs 38%; p=0.04] and proteinuria [3.4 (2.5-4.7) vs 2.0 (1.1-3.5) g/24hr; p<0.05] compared to non-Caucasians. No differences were noted for baseline HbA1c, cholesterol, systolic BP and duration of diabetes and proteinuria. In a Cox multivariate analysis, Caucasians had a hazard ratio (95% CI) of 1.9 (1.2-3.0); p=0.005, for proteinuria, which was independent for age [1.0 (0.9-1.1)], HbA1c [1.8 (0.4–9.4)], CAD [2.0 (0.9-4.5)] and baseline creatinine [2.6 (1.3-5.2); p=0.01]. In a similar analysis for non-Caucasians none of the risk factors were significantly related to risk of mortality. After adjusting for ethnicity, age, HbA1c, CAD, and baseline creatinine, baseline proteinuria was still associated with mortality [HR = 1.4 (1.1-1.9); p=0.02]. **Conclusion:** Proteinuria in type 2 diabetes is associated with a poorer prognosis regardless of ethnicity. The worse renal state at presentation in Caucasians is ill-understood, and needs further study.

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DECLINING INCIDENCE OF NEPHROPATHY (BUT NOT RETINOPATHY) IN TYPE 1 DIABETIC SUBJECTS IN CANTERBURY, NEW ZEALAND.

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Aim: The incidence of nephropathy in type 1 diabetes is declining in some but not all populations. Our aim was therefore to investigate whether there has been any change in the incidence of nephropathy (and retinopathy) in the Canterbury, New Zealand population and whether this can be related to glycaemic control or other factors.
Methods: We identified 265 subjects with type 1 diabetes (and age of diagnosis < 20 years) from a population-based register which was originally established in 1984. Subjects were divided into three 7-year groups according to year of diagnosis; 1970-76 (n=73), 1977-83 (n=90) and 1984 onwards (n=102). The time of onset of nephropathy (onset of persistent reagent-strip positive proteinuria or albumin ≥ 300mg/1) or diabetic retinopathy (any clinical grade) was established from clinical records.
Results: Cumulative incidence (SE) of nephropathy at 15 years was 10.5(3.3)% for the 70-76 group and 4.8(10.7)% for the 77-83 group. At 20 years, cumulative incidences were 24.1(5.4)% for the 70-76 group and 12.7(19.4)% (by extrapolation) for the 76-83 group. At 25 years (by extrapolation), cumulative incidences were 40.5(7.9)% and 24.8(23.8)% for the 70-76 and 77-83 groups respectively. For retinopathy, cumulative incidences at 10 years were 22.5(4.4)% and 29.4(5.7)% for the 70-76 and 77-83 groups respectively and (by extrapolation) 26(5.8)% for the 84 onwards cohort. By 25 years (by extrapolation), cumulative incidences of retinopathy were 97.6(1.7)%, 98.3(1.5)% and 99.9(1.7)% respectively. For successive groups, the mean (SE) HbA1c (available from 1985) for all results were 7.7(1.4)%, 7.9(1.9)% and 7.6(1.6)% respectively, NS.
Conclusions: The incidence of nephropathy, but not retinopathy is therefore declining. Glycaemic parameters are well matched between the 3 groups. It is postulated, therefore that other factors such as more aggressive blood pressure screening and management are responsible for the observed fall in the incidence of nephropathy.

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COURSE OF NEPHROPATHY IN TYPE-2 DIABETES IN BANGLADESH

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Aim: To evaluate the course of clinically defined diabetic nephropathy (DN) and clinically suspected non-diabetic glomerular disease (NDGD) in Bangladeshi patients with type-2 Diabetes (DM). **Material & Method:** Consecutive 432 overt proteinuric type2 DM patients (m=260, f=172) with bilateral symmetrical renal involvement attending dedicated renal clinic for diabetic patients between October 1997 to August 1998. Mean (±SD) age was 55.2±10.2 yr. and mean known duration of DM was 9.5±7.2 yr. Diagnosis of DN & NDGD was based on presence/absence of diabetic retinopathy and renal biopsy in selected cases. **Result:** Of the studied subjects, 261 had DN and 171 had NDGD. NDGD was more frequently found in female patients than male (47.1% vs. 34.6%, p=0.013). Duration of DM was longer in DN (10.7±7.5 vs. 7.6±6.3 yr., p<0.001). Proteinuria was also higher in DN (2.4±2.1 vs. 2.0±2.0 gm/day, p=0.049). About one-third patients (DN=75, NDGD=65) presented with nephropathy at the time of detection of diabetes. For the rest (DN=186, NDGD=106) the time elapsed to develop nephropathy (TN) varied from 2 to 33 yr. (median 8 yr.). TN was shorter in NDGD than DN (8.2±5.6 vs. 10.3±7.0 yr., p=0.009), also in female subjects than in male (7.6±4.6 vs. 10.8±7.4 yr., p<0.001). TN was shorter in patients who developed hypertension (HTN) before the age of 45 yr. (7.5±4.2 vs. 10.6±7.6 yr., p=0.002), also in patients who developed DM after the age of 50 yr. (6.8±4.3 vs. 10.6±7.1 yr., p<0.001). Female patients developed HTN much earlier than their male counterparts (45.2±9.1 vs. 52.9±11.3 yr. of age, p<0.001). These factors were also significant in multivariate analysis. However, female sex lost the significance when age of onset of HTN was considered in regression model, suggesting that comparatively early appearance of HTN in female subjects might predispose them to the early development of nephropathy in the course of type2 DM. **Conclusion:** In Bangladeshi subjects with type-2 DM, the course of NDGD differs from that of DN by: female preponderance, low grade proteinuria & early development of nephropathy. Appearance of hypertension at early age, onset of DM at late age, presence of NDGD and female sex are associated with early development of nephropathy in type -2 DM.

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INSULIN LISPRO LIMITS POST-PRANDIAL HYPERGLYCEMIA AND HYPERFILTRATION IN TYPE 2 DIABETES

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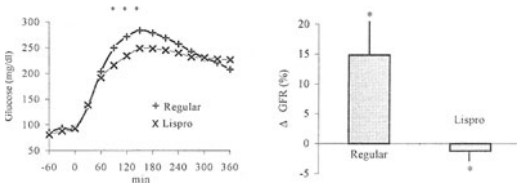
Aims: To evaluate post-prandial effects of Regular and Lispro Insulin in Type 2 diabetics with nephropathy. **Materials and Methods**: In 11 type 2 diabetics [age: 61.5 (50-72) years; serum creatinine: 1.1 (0.9-1.6) mg/dl; albuminuria: 563.0 (26.2-2534.6) µg/min], we evaluated two weeks apart the effect of subcutaneous Regular or Lispro Insulin (fixed dose: 0.1 U/kg body weight) given after a 2-hour euglycemic clamp and, respectively, 30 and 5 min before a standard meal (692 Calories: 54.2% Carbohydrates, 17.4% Proteins, 28.4% Lipids) on 6-hour post-prandial blood glucose, insulin and C peptide profiles. Euglycemic and post-prandial GFRs (inulin renal clearance) were compared on each study. Patients were fasting and without antidiabetic therapy from the evening before. Comparisons were by Student t-test. Data were as follows:



Results: Lispro, as compared to Regular Insulin, significantly limited blood glucose increase from 90 to 210 min after meal because of a prompter absorption which, combined to a faster clearance from the circulation, also avoided late hypoglycemia. Limiting hyperglycemia completely prevented the acute post-prandial GFR increase that consistently followed Regular Insulin administration. C peptide profiles were identical after the two insulin administration.

Conclusions: In diabetes, chronic Lispro therapy, by preventing glucose-induced hyperfiltration, could be renoprotective in the long-term.

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INFLUENCE OF GENDER AND ACE-INHIBITION ON THE COMPLICATIONS PROFILE OF TYPE 1 DIABETIC PATIENTS DURING A 5 YEAR FOLLOW-UP.

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Aims: Study on the impact of a 5-year education and treatment-programme on the chronic complications profile of type 1 diabetic patients. Methods: Diet, exercise and insulintherapy were optimized on the basis on an individualized self-bloodglucose monitoring system. A standardized complications profile was made on a yearly basis in order to detect and stage retinopathy, nephropathy and neuropathy. Patients with hypertension (>140/85 mm Hg) were treated with Ace-inhibitors in an individually adapted dosage. Patients : The cohort consited of 203 patients (124 men and 79 women) with a median age of 39 y (range : 19-71) and a duration of 12 y (range :1-51). The women were slighty but significantly younger (37 versus 40.5 y in men, p<0.02) but the duration of the disease was identical in both groups. Results : After 5 years the mean HbA_{1c} decreased in men from 8.3%, sd 1.1, to 8%, sd 1, (p<0.005) and in women from 8.3%, sd 1.2, to 7.8%, sd1.1, (p<0.001). At the start of the study, 39% of the patients showed signs of incipient or established nephropathy. After 5 years this figure was reduced to 18% (p<0.0001) for the whole population, but the reduction was more impressive in women (10% vs 23% in men). The treatment with Ace-inhibitors in some patients had only a modest influence on this evolution (p<0.05). In 12 patients (10 men and 2 women) there was a worsening in the stage of nephropathy accompanied by a non significant rise of the HbA_{1c} from 8.4% to 8.7%. Neuropathy was present in 53% of the population and this figure did not decrease significantly after 5 years. At this moment however, more women than men maintained a normal EMG: 74.5% vs 53% (p<0.01). Treatment with Ace-inbitors did not change the evolution of the neuropathy. After 5 years there was a significant decrease of the number of patients maintaining a normal eye-fundus (88 versus 118 at the start, p<0.005). Despite the better metabolic control, this observation was more pronounced in women (37 versus 51, p<0.04) than in men (51 versus 67, ns). In both sexes the addition of Ace-inhibitors improved the stage of retinopathy significantly (OR=6.205,95% CI :2.45-15.7, p<0.0005). Conclusions : In regard of metabolic control, it looks that a structured education and treatment programme for type 1 diabetic patients is more successfull in women with a better prognosis in regard of the development of nephropathy and neuropathy, but not retinopathy. Treatment of hypertension with Ace-inhibitors can improve the prognosis of retinopathy.

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ERYTHROPOIETIN DEFICIENT ANAEMIA OF EARLY DIABETIC NEPHROPATHY

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Aims: To determine whether diabetic patients with early established nephropathy are anaemic and whether this is due to erythropoietin (EPO) deficiency. **Materials And Methods:** Type 1 diabetic patients, mean age 41 years (range 28-59), with persistent proteinuria (minimum 280mg/D, mean 1719 ± 1335mg/D), creatinine 95 ± 27umol/L (maximum 157 umol/L) and diabetic retinopathy were screened for anaemia, serum EPO was determined and other causes of anaemia excluded. EPO levels were compared with values obtained from a group of 23 non-diabetic patients with iron deficiency anaemia. **Results:** 9 out of 20 diabetic patients with early nephropathy were anaemic with Hb 10.9 ± 0.8g/dl (mean ± SD) with serum EPO concentration of 8.3 ± 5.0 IU/L, (minimum < 2.5 IU/L).The iron deficient group (Hb 9.8 ± 1.4 g/dl) had a significantly higher serum EPO concentration (64.1± 41.2 IU/L, p < 0.01), for a similar degree of anaemia. **Conclusion:** This study has shown that the presence of early diabetic nephropathy is sometimes associated with an EPO deficient normochromic normocytic anaemia, which is expected only in more advanced renal failure. EPO deficiency may be due to damage of the renal interstitial tissue, the site of EPO production.

PS 90

Coronary Vascular Disease

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INCREASED CORONARY HEART DISEASE ASSOCIATED WITH INSULIN RESISTANCE IN POPULATIONS WITH & WITHOUT TYPE 2 DIABETES

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Aims: This analysis estimates the annual coronary heart disease (CHD) events attributable to insulin resistance (IR) in the US among those with and without type 2 diabetes mellitus (DM2). In addition, this study applies national cost data to these figures to develop an estimate for the annual cost of CHD attributable to IR. **Materials and Methods:** A literature search was conducted of Medline-indexed articles with information on IR and its association with heart disease. Data were sought from the identified articles, for populations with and without diabetes, on the prevalence of IR, the relative risk (RR) of CHD relative to IR status, and the number of CHD events per year expected in these two groups. These data were used to develop estimates for the number of CHD events per year by IR and DM2 status, the proportion of these events that are attributable to IR, and estimates for the annual cost of CHD events. **Results:** The prevalence of IR was found to be 92% among those with DM2 and 12% among the general non-DM2 population. IR increases the risk of a CHD event almost twofold (RR=1.95) in the DM2 population and 2.2-fold (on average) in the non-DM2 population. Of the 1.1 million annual CHD events in the US, 84% occur in those without DM2, while the remainder occur among those with DM2. Of the 176,000 annual CHD events in the DM2 population, 169,000 (96%) occur in those with IR, while 181,000 of the 924,000 annual CHD events in the non-DM2 population occur among those with IR. Calculations of attributable risk estimate that 82,000 of the annual CHD events in those with DM2 and 99,000 of the annual CHD events in those without diabetes are attributable to IR. Thus, IR is responsible for 46.6%, 10.1%, and 16.5% of the annual CHD events in the DM2, non-DM2, and total US population, respectively. These CHD events will result in an estimated annual total cost of $16.44 billion in the US in 1999, of which $8.75 billion are direct medical costs. **Conclusions:** A large number of annual CHD events and their subsequent costs can be attributed to IR. Preventing or modifying IR would offer substantial benefits in terms of decreasing the morbidity, mortality, and costs associated with CHD.

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PLASMA SOLUBLE ADHESION MOLECULES (VCAM-1, ICAM-1 AND E-SELECTIN) IN INSULIN DEPENDENT DIABETES MELLITUS

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Aims: The prevalence of cardiovascular complications is high in IDDM, especially if incipient or overt nephropathy is present. Adhesion molecules; VCAM-1, ICAM-1 and E-selectin are upregulated in the vascular endothelial cells in response to atherogenic stimuli and play a central role in the atherosclerotic process. This study investigates the impact of early diabetic nephropathy on plasma concentrations of adhesion molecules aiming to illustrate factors of potential pathophysiological relevance for the excess cardiovascular morbidity.
Materials and Methods: In total 56 IDDM patients, m/f=33/23, aged 47±8 (mean±SD) divided in 4 groups with either normoalbuminuria (n=16), microalbuminuria (n=15), macroalbuminuria (n=13) or macroalbuminuria combined with an elevated s-creatinine of 0.120 to 0.350 mmol/L (n=12) were included and compared to 16 healthy controls. The groups were comparable with regard to age, sex, smoking habits, BMI, diabetes duration and age at onset. Concentrations of adhesion molecules were measured by ELISA methods and the results logarithmically transformed before statistical analyses.
Results: VCAM-1 and ICAM-1 concentrations were similar in healthy controls and normoalbuminuric IDDM patients but concentrations of both molecules increased significantly with advancing diabetic nephropathy, p=0.002 and p=0.0009, *ANOVA*.

	controls	normoalb.	microalb.	macroalb.	s-crea.↑
VCAM-1	588(476-887)	598(490-968)	675(485-852)	680(542-1088)	852(498-1318)
ICAM-1	211(142-434)	235(174-370)	304(197-521)	341(224-571)	324(229-475)

Advancing nephropathy was associated with deterioration of glycaemic control and the difference in VCAM-1 concentration between the groups was only of borderline significance, p=0.05, if HbA1c was included as covariant, *ANCOVA*. However, the association between ICAM-1 and advancing nephropathy was intact, p=0.008.
Conclusions: Plasma concentrations of VCAM-1 and ICAM-1 are elevated in IDDM patients with early diabetic nephropathy, which can be of pathophysiological importance for the excess cardiovascular risk in these patients.

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TYPE 2 DIABETES MELLITUS IS ASSOCIATED WITH MULTIVESSEL CORONARY ATHEROMA IN UK ASIANS.

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Aims: To determine whether Asian patients with type 2 diabetes mellitus have differing degrees of stenosed major coronary artery systems at coronary angiography than Asian patients without diabetes mellitus. **Materials and Methods:** 136 Asian patients attending for elective coronary angiography at four centres in West Yorkshire, UK, were recruited. Diabetes and history of myocardial infarction were defined by WHO criteria. The angiogram results were graded by number of major coronary artery systems containing stenoses of 50% or greater. **Results:** 58 patients (42%) had type 2 diabetes. The two groups of patients were of similar age (mean age 55.3 yrs [no DM] vs 57.8 yrs [DM]), and sex distribution (86% male [no DM] vs 84% male [DM]). Smoking frequency (16% [no DM] vs 13% [DM]), and history of previous myocardial infarction (50% [no DM] vs 54% [DM]) were similar in both groups. Mean blood pressures (134/80mmHg [no DM] vs 139/80mmHg [DM]), mean total cholesterol levels (5.2 mmol/l [no DM] vs 5.4 mmol/l [DM]), and mean fibrinogen levels (3.17g/l [no DM] vs 3.26g/l [DM]), were similar in both groups of patients. Patients with diabetes showed a greater frequency of involvement of 2 or more major coronary artery systems (p=0.002).

		No history of diabetes		Type 2 diabetes	
		Number	%	Number	%
Number of major	0	20	26	4	7
coronary artery	1	28	36	14	24
systems with 50% or	2	12	15	19	33
greater stenoses	3	18	23	21	36

Conclusion In UK Asians with type 2 diabetes mellitus, more coronary artery disease is found at coronary angiography despite similar levels of other conventional risk factors. It is uncommon to find normal coronary arteries at angiography in Asians with diabetes.

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CORONARY REVASCULARISATION IN A 10 YEAR STUDY OF 385 TYPE 2 DIABETIC PATIENTS.

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Aims. The aims were to study the influence of invasive intervention on clinical outcome in type 2 diabetic patients with signs of atherosclerotic heart disease. **Materials and Methods.** The study population consisted of 385 type 2 diabetic patients with an age at diagnosis $\geq$ 30 years, who were followed for 10 years or until death. The age at entry was 54$\pm$10 years and the diabetes duration 8$\pm$7 years (mean$\pm$SD). Odds ratios were calculated in relation to whether the patients were treated with coronary by-pass surgery or angioplasty (PTCA). **Results.** Out of the 385 patients included, 108 patients were identified as having clinical signs of atherosclerotic heart disease. Out of these 108 patients, 30 patients had had a coronary angiogram out of whom 23 underwent coronary by-pass surgery or PTCA. Among patients who were revascularised, 4 patients died during the observation period compared with 57 out of 85 patients who did not undergo revascularisation (p<0.001). Among 68 patients who had had a myocardial infarction during the observation period, 11 had undergone revascularisation, out of whom 4 died, compared with 46 patients who died out of 57 patients with myocardial infarction who were not revascularised (p<0.001). **Conclusion.** In type 2 diabetic patients, the risk of death was clearly reduced in patients undergoing coronary by-pass or PTCA. Despite this fact, only a minority of the patients (21%) were treated with active coronary intervention. Thus, there is a potential for improved survival in this patient group with a more active treatment strategy

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LEPTIN AND OTHER RISK FACTORS IN MEN WITH CORONARY ARTERY DISEASE AND DISTURBANCES OF CARBOHYDRATE METABOLISM.

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Recently a great attention has been paid on leptin role in metabolic disturbances and some data suggest that hyperleptinemia rather than hyperinsulinemia may be a key factor in the pathogenesis of metabolic syndrome X.

Aims:The aim of our study was the analysis of serum leptin concentrations and other known risk factors (insulin, total cholesterol, LDL and HDL - cholesterol, triglicerides, fibrinogen) in men with coronary heart disease (CAD), referred for coronary arteriography.

Materials and Methods: 111 men (mean age 52,7 ± 9,1; mean BMI 27,9±3,5 kg/m2) with clinical symptoms of CAD and positive exercise test were included in the study. Coronary arteriography showed one vessel disease in 39 patients, two vessel disease in 35 patients, and three vessel disease in 28 subjects; 9 subjects had not any significant changes in coronary arteries. An oral glucose tolerance test with glucose and insulin estimations was performed in all patients. Serum leptin and insulin concentrations were measured using RIA method. ANOVA and Mann-Whitney test were used for statistical analysis.

Results: Serum leptin concentration did not differ significantly between subjects with normal (7,25 ± 4,7 ng/ml) and impaired glucose tolerance (8,48 ± 6,8 ng/ml) or diabetes (7,12 ± 2,8 ng/ml). However, the patients with 1-, 2- or 3- vessel disease had slightly higher leptin levels (7,58 ± 6,4 ng/ml, 8,73 ± 5,7 ng/ml and 7,05 ±4,0 ng/ml, respectively) in comparison with men without stenosis of coronary arteries (5,82 ± 2,6 ng/ml). The men with CAD had also significantly higher concentration of total and LDL - cholesterol, triglicerides, fibrinogen and HbA1c, and markedly lower ejection fraction and the level of HDL - cholesterol. Significant correlation between leptin concentration and BMI (r=0,5812, p<0,0001), post load insulin level (r=0,2850, p<0,01) and blood platelets count (r=-0,2518, p<0,05) were found.

Conclusion: Our results suggest that serum leptin concentration can not be considered as a direct risk factor of coronary artery disease.

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CORONARY BLOOD FLOW ASSESS WITH THE USE OF SINGLE PHOTON EMISSION COMPUTER TOMOGRAPHY (SPECT) IN PATIENTS WITH TYPE I DIABETES AND NEGATIVE ECG EXERCISE TEST

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Long history of diabetes and presence of diabetic microangiopathy significantly increase the risk of coronary heart disease. A different clinical cause, especially painless coronary episodes, explain an earlier cardiological diagnostic procedures in this group of patients. **Aims:** the aim of this study was to assess the coronary blood flow with the use of single photon emission computer tomography (SPECT) in patients with diabetic history longer than 5 years and with negative ecg exercise test. **Materials and methods:** the study was performed in group of 15 Type I diabetic patients (6 male, 9 female aged 28.6 ±6.6 years, diabetes history 13.6 ± 3.9 years, HbA1c 8.5 ±1.7%) and with negative history towards heart diseases. 6 of our patients had microalbuminuria. Myocardial perfusion was studied with single photon emission computer tomography (SPECT) at rest and during ischemia induced pharmacologically with dipiridamol (0.56 mg/kg body weight). We used MIBI (methoxy-isobutyl-isonitrile) marked with ^{99m}Tc in dose 2x20 mCi. **Results:** in 6 patients (40%) the SPECT showed normal blood flow both at rest and after dipiridamol. 7 patients (46.7%) showed decreased radioactivity in the dipiridamol test while it was normal at rest. In two cases (13.3%) dipiridamol increased disturbances in myocardial perfusion that were already present at rest. In 55.5% of cases abnormal perfusion coexisted with microalbuminuria. **Conclusions:** 1. Most of Type I diabetic patients, with no complaints and negative ecg exercise test, show disturbed perfusion evaluated by SPECT. 2. Long history of diabetes and/or microalbuminuria seem to indicate the necessity of further precise evaluation

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ISCHEMIC REACTION IN DIABETIC PATIENTS WITH CORONARY HEART DISEASE TREATED WITH GLIBENCLAMIDE OR GLIMEPIRIDE

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Aims: Sulfonylurea compounds, like glibenclamide, are known to interact with myocardial and vascular ATP-sensitive potassium channels. The aim was to compare the influences of glimepiride, a newly developed sulfonylurea, which is assumed to have less potential for cardiac effects by acting more specifically on pancreaticl receptors, and glibenclamide on myocardial perfusion. **Materials and Methods:** Six Type 2 diabetic patients (4 m / 2 f), age 66 ± 7 y., BMI 27 ± 3 kg/m^2, 11 ± 5 years after diabetes onset, HbA_{1c} 8,7 ± 2.1 %, with known coronary heart disease (history of myocardial infarction) and glibenclamide treatment (9.3 ± 1.8 mg/d) for 7,0 ± 5.8 years underwent two myocardial szintigraphic scans: The first under glibenclamide therapy and the second 14 days after changing to glimepiride (3.0 ± 1.1 mg/d) (One-day-protocol: ^{99}Tc-MIBI, 250 MBq at rest and 750 MBq after physical or pharmacological stress, SPECT > 1h after each injection). 25 segments of each scan were assessed semiquantitatively (0 = no perfusion to 3 = unrestricted perfusion). The scores of all 25 segments were summed up for resting- and stress-conditions. Differences were calculated. Statistics: Student´s t-test. **Results:** The perfusion score for resting-conditions was 67 ± 8 vs. 64 ± 8 (glibenclamide vs. glimepiride; p = 0.57). For stress-conditions it was 58 ± 9 vs. 56 ± 11, p< 0.01 versus resting conditions in both scans, p= 0.61 for the comparison of glibenclamide vs. glimepiride. The differences in scores between resting and stress (9 ± 6 vs. 8 ±7 points; p = 0.78 also was not significant. **Conclusions:** There is no significant difference in the myocardial ischemic reaction to stress in treatment with the sulfonylureas glibenclamide or glimepiride in Type 2 diabetic patients with coronary heart disease.

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PROGNOSTIC VALUE OF SILENT MYOCARDIAL ISCHEMIA IN DIABETIC PATIENTS.

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Data concerning the predictive value of silent myocardial ischemia (SMI) in diabetic patients are scarce. **Aims** : to examine, in a multicenter study, the predictive value of SMI for major cardiac events (MCE) in a large cohort of diabetic patients free of cardiac symptoms. **Materials and methods** : SMI was assessed on myocardial scintigraphy and a stress test when it was possible in 451 diabetic patients, 50 type 1 and 401 type 2, with at least one additional cardiovascular risk factor. Patients have been followed during 5.5 ± 1.1 (SD) years. **Results** : MCE defined by myocardial infarction, sudden death or the secondary requirement of myocardial revascularization procedure occurred in 61 patients. Among the patients with SMI the rate of MCE was significantly higher than in patients free of SMI (23.2% vs 9.1%, p < 0.0001), with an Odds ratio = 3.02 (95% CI : 1.72-5.29). The occurrence of MCE was significantly associated with age (r = 0.163, p = 0.001). The analysis according to the Kaplan Meyer method confirms the significant association between SMI and MCE (p = 0.0002) after adjustment on the center. With age and center taken as independent parameters, the Cox model confirms the significant association between SMI and MCE : Odds ratio = 2.28 (95% CI : 1.27-4.09). **Conclusion** : this multicenter study 1) confirms that SMI has a high predictive value of MCE ; 2) strongly suggests that SMI should be assessed in diabetic patients with other cardiovascular risk factors.

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DOBUTAMINE STRESS ECHOCARDIOGRAPHY FOR THE DIAGNOSIS OF SILENT ISCHAEMIA IN DIABETIC PATIENTS.

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Aims: silent myocardial ischaemia (SMI) is frequent in diabetic patients (pts). The aim of this study was to determine the value of Dobutamine stress echocardiography (DSE) for the diagnosis of SMI, compared to exercise stress testing (ST) and to Thallium myocardial scintigraphy (Th). **Material and Methods**: 50 diabetic pts, with a known duration of diabetes > 15 years for type 1 and > 5 years for type 2, having at least 3 added risk factors (hypertension, cigarette smoking, dyslipidemia, other vascular disease, micro/macroalbuminuria, family history of premature CAD), but neither chest pain, nor EKG abnormalities, were enrolled in this prospective study. All of them were submitted to DSE with a maximum infusion rate of 40 γ/kg/mn Dobutamine ± 1 mg IV Atropine, and to Th coupled with bicycle stress testing. Coronarography was performed if at least one exam was abnormal. **Results**: preliminary results concerning 50 pts (34 M, 16 F, average age 59.8 ± 10 years, 10 type 1 and 40 type 2 diabetes, known duration of the disease 17.1±9.3 years) were analysed. Feasibility of DSE was 94 % despite frequent obesity; no serious complication occurred during the test. Coronarography was performed in 22 pts (44 %); 13 were abnormal (26 % of the whole group): 5 pts had a single-vessel, 4 a 2-vessel and 4 a 3-vessel disease. Predictive positive value was 71.5 % for DSE vs 60 % for Th and 40 % only for ST. DSE was falsely negative in 3 cases vs 7 cases for Th and ST.

Conclusion: asymptomatic coronary disease is common in diabetes associated with other risk factors. DSE appears promising for its detection and a good alternative to Th.

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CARDIOVASCULAR RISK FACTORS IN TYPE 1 DIABETIC PATIENTS WITH MANIFESTATION AFTER 35 YEARS (LADA) COMPARED WITH TYPE 2 DIABETICS

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The aims of the study were to compare cardiovascular risk factors in patients suffering of type 1 diabetes with manifestation after 35 years of age (LADA) with the age, sex and duration of diabetes group of type 2 diabetic patients (DM2).**Materials and methods**: LADA was diagnosed according to following criteria: manifestation of diabetes after 35 years of age, fasting C peptide less than 200 pmol/l and therapeutic dependency on insulin. Criteria for DM2 were: fasting C-peptide more than 200 pmol/l, antibodies against glutamate decarboxylase (GADA) less than 50 ng/ml and no necessity of insulin therapy. LADA group consisted of 39 patients (16 men and 23 woman) with the average age of 63,4±14,5 ys and average duration of diabetes of 15,6±10,0 ys. All of the patients were treated by insulin. The DM2 group consisted of 54 patients (22 men and 32 woman) with the average age of 66,76 ys and average duration of diabetes 7,9±7,4 ys Patients of both groups did not differ in the age and sex duration of diabetes of LADA patients was significantly longer (p<0,001) In each person family history of myocardial infarction and diabetes was recorded, the arterial blood pressure was taken and plasma levels of total and HDL cholesterol, triglycerides and uric acid were measured. **Results:** Family history regarding diabetes and myocardial infarctions in mothers and fathers did not difffer between LADA and DM2 group. Systolic and diastolic blood pressure did not differ in both groups (sBP 136±21 vs. 142±22mmHg, dBP 78±10 vs. 82±11 mmHg ns.). The levels of total plasma cholesterol also did not differ among the groups (LADA 4,92±0,52 mmol/l and DM2 5,380±0,57 mmol/l ns.) HDL cholesterol was significantly higher in LADA group (1,58±0,15 mmol/l vs.1,19± 0,10 mmol/l p<0,001), plasma triglycerides were higher in DM2 group (1,44±0,19 vs. 1,79 ±0,34mmol/l, p<0,05). Plasma uric acid levels were also higher in DM2 group (212,8 ±37,2 vs. 270,4±35,4 umol/l, p<0,01). **Conclusions** Even the diabetes duration was significantly longer in LADA group, our data demonstrates moderately lower cardiovascular risk in LADA group as compared with group of type 2 diabetic patients of comparable age and sex.

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Cardiovascular Complications – Treatment and Signs

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VITAMIN SUPPLEMENTATION REDUCES OXIDATIVE STRESS IN PATIENTS WITH TYPE 2 NON-INSULIN DEPENDENT DIABETES

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Aims: We have previously demonstrated increased oxidative damage in elderly type 2 diabetic patients with vascular complications despite similar antioxidant defences as a healthy control group. These patients may require greater antioxidant defences than normal elders to protect against oxidative damage. We therefore assessed markers of antioxidant defence and oxidative damage in patients with type 2 diabetes before and after supplementation with low and high doses of a combination of the major water- and lipid-soluble vitamins C and E. **Materials and Methods:** Nine elderly type 2 diabetic patients with cardiovascular complications (mean age±sem 76.9±2) took a low dose combination of vitamins C (500mg) and E (400IU) for 4 weeks. Following a 4-week washout period, patients had a further 4 weeks of supplementation with high doses of vitamin C (1000mg) and E (800IU). Blood was sampled pre- and post-treatment for analysis of total antioxidant capacity (TAC) by enhanced chemiluminescence, vitamin E by HPLC, and plasma lipid hydroperoxides (LHP) (the marker of oxidative damage) by colour spectrophotometry. Statistical analyses were performed using analysis of variance (ANOVA). **Results:** Vitamin E was significantly increased compared with baseline, particularly after the high dose combination (59.8±6 vs. 36.4±4, p<0.001 (low) and 72.7±11 vs. 30.8±5, p<0.001 (high)). TAC was significantly increased above baseline to a similar degree after both doses (508.2±33 vs. 436.4±31, p<0.01 (low) and 519.3±48 vs. 440.8±34, p<0.01 (high)). In contrast LHP was reduced to a greater degree after the low dose combination (6.1±1 vs. 12.1±2, p<0.01 (low) and 8.0±1 vs. 11.6±1, p<0.05 (high)). **Conclusions:** We have demonstrated that low dose supplementation with the antioxidant vitamins C and E can significantly increase antioxidant defences and reduce oxidative damage in elderly patients with type 2 diabetes. This study has helped to define a regimen which could now be evaluated in clinical trials.

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SERINE-PROTEASE INHIBITORS IN DIABETIC CARDIOMYOPATHY.

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Diabetes mellitus is characterized by early and rapidly progressive atherosclerosis. In diabetic patients, in addition to myocardial ischemia, microaneurisms and interstitial damage have been described. The pathogenesis of such lesions is unclear. Alpha-1-antitrypsin and antithrombin III, both serine-protease inhibitors, inhibit proteases such as elastase, trypsin, chymotrypsin and thrombin. These serine protease inhibitors function in hemostasis and in the remodelling of connective tissues, both of which have been inplicated in the pathogenesis of atherosclerosis. **Aim:** we set out to evaluatae the role of these two serine-protease inhibitors in diabetic heart disease. **Materials and Methods**: in this prelimenary investigation, we studied myocardial tissues obtained by heart biopies from 27 normal hearts from transplant donors, 4 patients undergoing heart valve surgery, 6 non-diabetic patients undergoing cornary bypass, and 6 diabetic patients undergoing bypass surgery. Our methodology employed standardized immunocytochemical procedures with monoclonal antibodies to the two serine-protease inhibitors, and the protocol was designed to map the differences in the tissue localization of these inhibitors in the different study populations by using the techniques discribed by Faulk and colleagues. **Results**: this study revealed that in normal control hearts the alpha-1-antitrypsin was located in the extracellular matrix and smooth muscle cells of large arteries and in the basal membrane of myocardiocytes, while the antithrombin III was located in the endothelium of veins and arteries as well as in the smooth muscle cells of large arteries. These reactivities were uniform in both their location and intensity of reactivity. In contrast, no inhibitor was uniform in its distribution in 5 of the 6 diabetic hearts, and a grading scheme for intensity revealed significant decrease in the 5 diabetic hearts. **Conclusions**: these results obtained from diabetic hearts with coronary artery disease differed qualitatively and quantitatively from those obtained from non-diabetic hearts with coronary artery disease. We suggest that the data from this small study suggest that serine-protease inhibitors function as inhibitors of thrombin-mediated damage to vascular endothelium and smooth muscle cells via antithrombin III, and as inhibitors of connective tissue damage via alpha-1-antitrypsin.

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IS SULFONYLUREA TREATMENT DETERMINATIVE ON PRECONDITIONING IN ALLOXAN DIABETIC RABBITS ?

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ATP-dependent K+ channels activity in the myocardiac cell is controlled by metabolic circumstances. Glyburide, the frequently used antidiabetic medicine is known as specific K+ channel blocking agent. So ischaemic activation of K-ATP channels and preconditioning of diabetic heart may be contraversial. **Aim**: In vivo studies were made on 5 control and 21 alloxan diabetic rabbits to asses if protective effect of ischaemiac preconditioning on myocardial infarct size is influanced by metabolic state and sulfonylurea treatment. The effects of three times of 2 minutes ischaemic preconditioning and clinical doses (0,02-0,1-0,2mg/kg) of Glyburide and Glimepirid on infarct size were compared by two ways of variancia analysis. **Method:** Infarcted areas in rate of left ventricule (TTC painting) and infarcted area/risk area (fluorescense signaled) ratios were assesed by planimetry (IBM PC, Iman 2,0 beta) and 2 ways of variancia analysis.. **Results**: While in healthy animals preconditioning diminished (48,8% - 29,6%, $p<0,05$), in diabetic subjects did not reduce (56,6% - 53,3%, p=NS) infarct size. In healthy animals Glyburide in all doses blocked protective effect of preconditioning (29,6% - 50,4% - 51,4% - 55,5%, $p<0,05$), but Glimepirid did not have such an influance (29,6% - 36,1% - 37,2%, p=NS, 43,4%, $p<0,05$). In diabetic subjects Glyburide in lower doses (53,3%-46,6%, $p<0,05$, 49,6% - 55,0%, p=NS) allowed some infarction limiting effects of preconditioning, while in case of Glimepirid treatment not the sulfonylurea effect, but the metabolic state seemed to be determinative. **Conclusion:** Pathological carbohydrate state prohibits protective effect of preconditioning in the myocardium. Both metabolic circumstances ($p<0,02$), sulfonylurea treatment ($p<0,05$) and preconditioning ($p<0,005$)posesses independent effect on infarct size. In the consequance of the common influance of metabolic state and sulfonylurea treatment, Glyburide seemed to be less harmful in diabetic circumstances than in healthy state.

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IMPROVED FIBRINOLYSIS WITH INTENSIVE INSULIN TREATMENT DURING AN ACUTE CORONARY EVENT IN DIABETICS.

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Aim: Many clinical and laboratory observations give support to the hypothesis that strict metabolic control by insulin infusion during acute coronary events may improve the ischaemic damage and prognosis. We investigate the impact of intensive insulin treatment on fibrinolytic parameters during an acute ischaemic myocardial event (unstable angina or acute myocardial infarction) in patients with type 2 Diabetes Mellitus .

Methods: The study group consisted of 48 type 2 diabetic patients. 24 randomized to conventional therapy plus intensive insulin treatment (group A) and 24 to conventional therapy only (group B) The two groups were comparable according to sex, age, bmi, whr, duration of Diabetes, previous antidiabetic treatment, type of ischaemic events, concomitant therapy and the classic risk factors for Coronary Heart Disease. Insulin treated patients were excluded from the study. Plasma levels of fibrinogen, t-PA and PAI-1 were measured on admission and discharge. Fibr was measured using photometric method. PAI-1 and t-PA were measured by enzyme-linked immunosorbent assays.

Results: Tissue plasminogen activator (t-PA) increased in both groups during hospitalization ($t\text{-}PA_{admission}$ vs $t\text{-}PA_{discharge}$, Group A:15 42±4.4 $ng.ml^{-1}$ vs 21.2±5.74 $ng\ ml^{-1}$ p=0.000037, Group B.14.47±6.31 $ng.ml^{-1}$ vs 19.18±6.88 $ng.ml^{-1}$ p=0,001). On the contrary, fibrinogen (fibr) and plasminogen activator inhibitor 1(PAI-1) levels increased remarkably in controls (Group B, $fibr_{admission}$ vs $fibr_{discharge}$: 2.98±1.04 $g.l^{-1}$ vs 3.59±1.01 $g\ l^{-1}$ p=0.002 and $PAI\text{-}1_{admission}$ vs $PAI\text{-}1_{discharge}$: 30.6±17.34 $ng\ ml^{-1}$ vs 40.62±23.48 $ng\ ml^{-1}$ p=0.003), finding that was not observed in intensive insulin treatment group (Group A, $fibr_{admission}$ vs $fibr_{discharge}$: 2.87±0.73 $g\ l^{-1}$ vs 2.67±0.72 $g\ l^{-1}$ p=0 101 and $PAI\text{-}1_{admission}$ vs $PAI\text{-}1_{discharge}$: 30.75±15.81 $ng.ml^{-1}$ vs 27.75±6.43 $ng.ml^{-1}$ p=0.484).

Conclusion: Intensive insulin treatment during an acute coronary event improves fibrinolytic profile in patients with Diabetes Mellitus. This is a possible mechanism for the reduced short and long term mortality in diabetic patients treated with intensive insulin treatment protocol.

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REVERSAL OF DIASTOLIC DYSFUNCTION AFTER PANCREAS TRANSPLANTATION IN DIABETIC UREMIC PATIENTS

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Aim. Epidemiologic studies have clearly demonstrated that diabetic patients are at increased risk for cardiovascular morbidity and mortality. Diastolic more than systolic function is frequently impaired in diabetes. Aim of our study was to evaluate the impact of glucose metabolism normalization on development of diabetic cardiomyopathy. **Materials and Methods.** We evalueted in 43 kidney-pancreas (KP) and in 27 kidney-alone (KA) diabetic uremic transplanted patients, the effect of pancreas tranplant on left ventricular diastolic function. As controls (C), 15 healthy subjects were choosen. Diastolic but not systolic dysfunction was present in 85% of KA and 81% of KP group. During the follow up patients were studied with a radionuclide assessment, with evaluation of ejection fraction (EF), peak filling rate (PFR), peak ejection rate (PER), time to PFR (t-PFR), PFR/PER ratio. Patients were grouped according to years of follow up (6 months: 15 KP and 9 KA; 2 yrs: 13 KP and 9 KA; 4 yrs: 15 KP and 9 KA). **Results.** At 6 months after tx, no differences have been shown between KP and KA, the two groups still presenting a slight isolated diastolic dysfunction, as shown by low PFR (KP=3.88±0.14 vs KA=3.93±0.28; ns but both $p<0.05$ vs C=4.16±0.32), reduced PFR/PER ratio (KP=0.97±0.04 vs KA=0.99±0.09, ns) and prolonged t-PFR (KP=162.6±5.3 vs KA=154.7±9.7, ns). At 2 years of follow up normal values of diastolic parameters were shown in KP, but not in KA group. At 4 years the differences between the two groups were significant for all diastolic parameters (PFR: KP=4.46±0.15 vs KA=2.73±0.24, $p<0.001$; t-PFR: KP=141.9±7.8 vs KA=209.4±13.5, $p<0.001$; PFR/PER ratio: KP=1.10±0.04 vs KA=0.81±0.08, $p<0.01$). Furthermore at 4 years, diastolic parameters were significantly different versus baseline group, demonstrating an amelioration of diastolic dysfunction in KP and a worsening in KA group. Finally, at 4 years a significant difference in systolic function was shown, with higher EF values in KP group than KA group (KP=75.7±1.86 vs KA=65.3±2.8, p=0.004). **Conclusion.** Normalization of blood glucose metabolism lead to improvement of diastolic function in IDDM.

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LEFT VENTRICULAR(LV) GLOBAL AND REGIONAL SYSTOLIC AND DIASTOLIC FUNCTION IN TYPE 1 DIABETES MELLITUS(DM)

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Patients with type 1 DM appear to have an increased incidence of early subclinical LV dysfunction. The aim of our study was to evaluate the global and regional LV function in young patients with type 1 DM. **Methods:** 44 patients with well-controlled type1 DM (group 1) and 27 healthy subjects with normal OGTT(group 2) were studied by using 2-D, pulsed and tissue Doppler echocardiography(TDE). LV ejection fraction(EF) and transmitral flow Doppler parameters (early peak diastolic-Emax and late peak diastolic velocity-Amax, global LV isovolumic relaxation time-IVRT and deceleration time-DT) were measured. LV myocardial wall velocities were recorded from the apical 4 chambers view in the basal segment of the lateral free wall (1) and the regional isovolumic relaxation time (IVRT1) and systolic (S1), early diastolic (E1) and late diastolic myocardial velocities (A1) were measured.

Results: Mean values +SD of the above parameters in group 1 and group 2 (ns not plated) are presented in table:

n=71	GROUP 1	GROUP 2	p value
Heart rate (bpm)	77+9	69+6	<0.001
EF(%)	69+3	65+2	< 0.001
Amax (cm/s)	60+15	49+9	< 0.05
S1 (cm/s)	17+2	12+1	< 0.001

Conclusion: 1) Global and regional left ventricular systolic function is increased in young patients with type 1 DM. 2) In these patients regional diastolic function, as assessed by TDE, is normal and the impaired LV diastolic function could be attributed to the higher heart rate.

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CARDIAC SURGERY-RELATED COMPLICATIONS IN DIABETES.

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Aims: Diabetes mellitus (DM) represents an additional risk factor for peri- and post-operative complications after cardiac surgery. Because of controversial data on this issue, probably due to not exactly comparable groups of patients, this retrospective study aimed to compare two highly homogeneous populations which were different only for the presence or absence of DM. **Material and Methods**: We studied 700 patients who underwent cardiac surgery in our hospital (between 1996 and 1998): 350 with and 350 without DM, aged 62 ± 9 y (67% males); 441 underwent coronary-aortic by-pass (CABP) and 259 valve operations (VO). DM accounted for 50% of the patients of both surgical procedures. Apart from DM, the two groups were strictly matched for age, weight, heart disease, blood pressure, concomitant pathologies (except for previous neurological injury more frequent in DM-group), and smoking habits. A good metabolic control in DM-patients was obtained. A number of intra- and post-operative complications or events (including death) were carefully evaluated. Univariate and multivariate analyses (Mann-Whitney U-test, chi-square test, stepwise regression and logistic regression models) were used. **Results**: DM-patients and controls showed a similar rate of mortality, but in DM-group more total neurological (3.5 fold risk, $p<0.03$) and renal (5 fold, $p<0.006$) complications, a higher (1.5 fold, $p<0.003$) reopening rate, more prolonged stay in intensive care unit ($p<0.006$), and a higher necessity of hemotransfusions ($p<0.006$) were found. Moreover, DM-patients who underwent VO showed a higher risk (5 fold, $p<0.03$) of major lung complications. **Conclusions**: DM does not seem to increase the mortality rate of cardiac surgery, in either CABP or VO procedures. However, in DM-patients renal and neurological complications, necessity of hemotransfusions and of reopening are significantly more frequent. In addition, DM-patients undergoing VO appear to be particularly prone to major lung complications.

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PROGNOSTIC VALUE OF GLYCAEMIA AFTER ACUTE MYOCARDIAL INFARCTION.

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Aims: To assess the prognostic value of glycaemia at admission on 28-day mortality after acute myocardial infarction (AMI).
Materials and Methods: In a prospective cohort of 662 consecutive AMI patients aged less than 75 years, glycaemia on admision and 4-days succesive fasting glycaemias were determined.
Results: 195 (30.5%) subjects had previously known diabetes mellitus, 457 (69%) showed a glycaemia above 120mg/dl on admission and 362 (59.9%) disclosed a mean glycaemia above 120mg/dl (kappa concordance was 0.65, correlation coefficient 0.88, $p<0.001$). The 28-days mortality was higher between patients with hyperglycaemia on admission (10.3%) compared with non-hyperglicemic patients (1.5%), $p<0.0001$. Higher mortality between patients with mean 4-day hyperglycaemia (9.1%) compared with patients with 4-days mean hyperglycaemia <120mg/dl (1.7%), $p<0.0001$ was observed. In a logistic regression analysis, the effect of glycaemia above 120mg/dl on admission on 28-day mortality was independent of comorbidity (risk coronary factors, including previous diagnosis of diabetes mellitus), severity (infarct characteristics) and therapeutic management (drug treatment, thrombolysis, percutaneous angioplasty) (OR= 3.93, 95% CI 1.1-13.9).
Conclusions: AMI patients with glycaemia above 120mg/dl at or during admission develop more lethal and severe AMIs than thouse without hyperglycaemia regardless of comorbidity, age and clinical characteristics.

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Hypertension

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CHANGES IN 24-H ABPM PROFILES IN PROSPECTIVE 3-YEAR OBSERVATION OF YOUNG TYPE 1 DIABETICS AND HEALTHY CONTROLS

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Aim: to investigate whether it is possible to find significant changes in blood pressure profiles in young diabetic (DM t.1) patients after relatively short, i.e. 3-year follow-up.
Materials and Methods: 23 non-microalbuminuric DM t.1 patients (13M, 10F, mean age 26±6 years, mean duration of diabetes 3.5±1 years) and 19 healthy controls (10M, 9F, mean age 26±3) underwent 24-hour ambulatory blood pressure monitoring (24-h ABPM). In both groups, 24-h ABPM recordings were repeated after 3 years. There were no significant differences between the two groups as for BMI, however it increased significantly in both groups during the 3 years of observation time.
Results: 24-h, daytime, as well as night-time means of systolic (SBP), and diastolic (DBP) blood pressure were not significantly different between DM t.1 patients and controls, both for the first and the second ABPM profiles. No significant changes in these variables were found, either in DM t.1 group or in control group, when paired comparisons (the first vs. the second ABPM profile) were performed. Table contains group means of repeated 24-h ABPM recordings in DM t.1 group and control group.

		DM t.1		controls	
		1st profile	2nd profile	1st profile	2nd profile
SBP-24h	[mm Hg]	120±8	122±8	119±9	118±9
DBP-24h	[mm Hg]	73±6	74±6	72±6	73±6
SBP-daytime	[mm Hg]	125±9	127±8	125±10	123±9
DBP-daytime	[mm Hg]	78±7	79±6	76±6	77±6
SBP-night-time	[mm Hg]	108±9	108±10	108±9	107±10
DBP-night-time	[mm Hg]	61±7	62±8	60±7	61±8

Conclusions: The prospective 3-year observation of patients with short-lasting DM t.1 and age, sex, BMI matched healthy controls revealed no significant differences in blood pressure levels estimated from repeated profiles. Further follow-up is necessary to establish the duration of diabetes when blood pressure control starts to differ between DM t.1 patients and healthy individuals.

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CUTPOINTS OF GLUCOSE ABNORMALITIES AND THE PREVALENCE OF HYPERTENSION

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Aim of the study was to investigate the prevalence of hypertension (HT) in patients classified according to their fasting plasma glucose (FPG) by the new ADA criteria.
Materials and Methods: Six hundred patients, consecutively admitted, were classified as NG: Normal Glucose (FPG<110mg%)148, IFG: Impaired Fasting Glucose (110-125) 91, DN: Diabetics according to New criteria (126-140)48, D:Diabetics(>140)313, divided in UD (Unknown Diabetics) 131 and KD(Known Diabetics)182. Patients under antihypertensive treatment or with B.P.≥140/90 were considered hypertensives.
Results: The prevalence of HT was found to increase progressively in the groups ranked according to the increment of hyperglycaemia. ($p<0.001$). HT was found in 56 of 148 (38%) in NG, 50/91 (55%) in IFG, 28/48 (58%) in DN and 202/313 (64.5%)in D, with 71/131 (56%) in UD and 131/182 (72%) in KD. Significant differences were observed between group N and the other groups (IFG, DN, D) taken either as a whole ($p<0.001$) or separately ($p=0.01$, <0.05, <0.001). No differences were found between IFG and each of the diabetic groups DN and D ($p>0.05$) or between IFG and both diabetic groups as a whole ($p>0.05$) as well as between these diabetic groups.
Conclusions: 1)Patients classified according to their FPG based on the new ADA criteria had an increasing prevalence of HT as their glycaemia increased. 2)The IFG group had a significantly increased prevalence of HT in relation to that with Normal Glucose but not different from the diabetic groups. 3)The FPG value of 110mg%, recently proposed by ADA as cutpoint between normal and abnormal glucose levels beyond than detected as a threshold of diabetic complications was also observed by us to be the threshold for increased prevalence of HT.

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No deleterious effects of tight blood glucose control on 24 h ambulatory blood pressure in normoalbuminuric IDDM patients.

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Background: Intensive therapy aiming at near normalization of glucose levels effectively delays the onset and slows the progression of complications in IDDM and is recommended in most patients. However, this approach was challenged in a recent report where intensive insulin treatment was found to be associated with deleterious effects on nocturnal blood pressure, the proposed mechanisms being subclinical nocturnal hypoglycemia or hyperinsulinemia. The aim of the present study was to evaluate the association between glycemic control, insulin dose, and 24-h ambulatory blood pressure (AMBP) in a group of well characterized IDDM patients.
Methods: 24-h AMBP was measured in 123 normoalbuminuric (UAE <20 µg/min) IDDM patients using an oscillometric technique (Spacelabs 90207) with readings at 20-minute intervals. UAE was measured by RIA and expressed as geometric mean of three overnight collections made within one week. HbA_{1c} was determined by HPLC (non-diabetic range 4.4-6.4%) and patients were stratified into quartiles according to HbA_{1c} levels. *Results:* Mean HbA_{1c} values in the four groups were 7.0% (n=31), 8.0%(n=31), 8.6%(n=31), and 9.7%(n=30).The groups were comparable regarding age, gender, diabetes duration, BMI, UAE, smoking status, and physical activity. AMBP levels were almost identical in the HbA_{1c} quartiles with night values of (increasing HbA_{1c} order): 110/63, 112/66, 112/66, and 113/65 mmHg (p=0.69/p=0.32). There was no association between tight glucose control and higher nocturnal blood pressure or a more blunted circadian blood pressure variation. On the contrary, a weak positive correlation between night/day ratios of mean arterial blood pressure and HbA_{1c} values was found (r=0.26, p=0.005) i.e. blunted circadian blood pressure variation is most frequent in patients with high HbA_{1c} values. Neither did we find doses of insulin to be associated with night blood pressure (r=0.04, p=0.68).
Conclusions: Tight blood glucose control is not associated with deleterious effects on 24 h ambulatory blood pressure in normoalbuminuric IDDM patients. Intensive therapy can be implemented without concerns of inducing high nocturnal blood pressure and accelerating diabetic complications.

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HYPERTENSION MANAGEMENT IN NIDDM PATIENTS WITH NEPHROPATHY

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Aims: 1. Prospective monitoring of blood pressure control and 2. Retrospective analysis of effect of combinations of anti-hypertensive agents (anti-HTN) on renal progression in hypertensive NIDDM patients with nephropathy. **Materials & Methods:** Consecutive 347 hypertensive, overt proteinuric NIDDM patients (m=209, f=138) with bilateral symmetrical renal involvement attending diabetic renal clinic between October 1997 to August 1998 who were followed-up for at least 6 months. Target blood pressure was =<130/80 mm Hg. Angitensin Converting Enzyme Inhibitor (ACE-I), calcium channel blocker (CCB), beta blocker, diuretics and alpha blocker were used according to set clinical guideline. ACE-I was the first drug of choice. **Results:** When the patients were referred to renal clinic, mean (± SD) systolic BP (sBP) was 147.9±23.34 and diastolic BP (dBP) was 86.5±11.10 mm Hg. Under nephrological care, achieved sBP (142.1±24.83) and dBP (76.5±10.46) were significantly (p<0.001) lower. Target dBP=<80 could be achieved in 297 (81.4%) cases, but the target sBP=<130 mm Hg was achieved in only 149 (40.8%) patients. One quarter of patients required one anti-HTN class, 28.2% required 2, 26.8% patients 3 and one fifth patients needed 4 or more anti-HTN classes. During mean follow-up period of 1.21±0.71 yr., in 182 (52.4%) patients mean serum creatinine decreased by 0.2 (95% CI 0.141 - 0.27) mg% and among the rest 165 (47.6%) patients, it increased by 1.2 (95% CI 0.970-1.44) mg%. In logistic regression model, only CCB was significantly associated with decline in renal function independent of use of other anti-HTN class (OR=1.97, p=0.005). It was also independent of number of anti-HTN and achievement of target sBP =<130 or dBP=<80 mm Hg (OR=1.90, p=0.04). However this unfavorable effect of CCB lost the significance (p=0.07) when achieved sBP=<125 mm Hg (but not =<130) was considered into the regression model, possible explanation being transmission of systemic pressure into the intraglomerular space with the use of CCB. **Conclusion**: The study (1) illustrates difficulty in controling systolic BP, (2) suggests caution for using CCB and (3) emphasizes reduction of sBP to =<125 mm Hg specially while using CCB alone or in combination to halt the progression of renal failure in NIDDM patients with nephropathy.

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ANTIHYPERTENSIVE THERAPY IN TYPE-II DIABETIC PATIENTS

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AIM: *To evaluate the efficacy of Monotherapy and combination of antihypertensive drugs in type-II diabetic patients.*

METHODS: *532 patients were randomised who visited the clinic atleast once in 2 months and were followed upto 3 years. Patients with secondary hypertension and those who were lost for follow up were excluded. ACE inhibitors, calcium antagonists, Beta blockers and Diuretics were used depending on their merits.*

RESULTS: *52(9.8%) who had preventable causes for hypertension were selected for non drug therapy. 198 out of 307(57.7%) who were selected for monotherapy were already on it at the time of study. 103 out of 156 (29.3%) and 12 out of 17(3.2%) were on combination of two and more than two drugs respectively. During the study it was observed that antihypertensive drug requirement proportionately increases with duration of diabetes. Patients on combination of drugs had fewer side effects and lesser incidence of related complications.*

Anti-HTN Therapy	*Beginning*	*One year*	*Two years*	*Three years*
Non-drug	*52(9.8%)*	*45(8.5%)*	*30(5.6%)*	*23(4.3%)*
One drug	*307(57.5%)*	*289(54.3%)*	*272(51.1%)*	*255(47.9%)*
Two drugs	*156(29.3%)*	*174(32.7%)*	*198(37.2%)*	*217(40.9%)*
3 or more	*17(3.2%)*	*24(4.5%)*	*32(6.1%)*	*37(6.9%)*

CONCLUSION: *Combination of anti-hypertensive drugs is beneficial and has additive effect in Type-II diabetic patients.*

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SALT-SENSITIVE BLOOD PRESSURE - AN INTERMEDIATE PHENOTYPE PREDISPOSING TO DIABETIC NEPHROPATHY ?

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Family studies point to important genetic determinants of diabetic nephropathy (DN). Blood pressure is higher in offspring of parents with type 2 diabetes and DN, but the patomechanisms involved have not been elucidated. We examined salt sensitivity of blood pressure (BP) after 5 days equilibration, on low (20 mmol/day) vs. high salt diet (low salt diet plus slow salt tablets to yield 220 mmol/day), respectively in 3 groups: (i) control individuals (n=10; 2 m, 8 f, mean age 36.6±7.1 y), (ii) offspring (n= 10; 2 m, 8 f; mean age 40.1±7.2 y) of type 2 diabetic parents without DN (DN-) and (iii) offspring of type 2 diabetic parents with DN (DN+) (4 m. 6 f; mean age 37.0±5.1). Ambulatory blood pressure was measured as were hormones involved in sodium homeostasis (PRA, aldosterone, ANP). Adherence to the diet was verified by measuring sodium excretion and Hct. In offspring of DN+ parents on low salt diet, systolic and diastolic BP was 128 ± 16.3/75.9 ± 9.3 vs. 121 ± 11.7/75.4 ± 9.1 in offspring of DN-parents (n.s.). On high salt diet, in offspring of DN + parents systolic and diastolic blood pressure was 135.4 ± 12.1/80.7 ± 8.2 mm Hg vs. 124 ± 10.2/75.0 ± 8.1 mm Hg in offspring of DN- (p.<0.01 for systolic BP). The BP difference in offspring of DN+ parents; i.e. of mean BP on high minus low salt diet, was 5.9 ±3.5 mm Hg vs. 0.2 ± 4.2 mm Hg in offspring of DN- (p<0.01). The proportion of salt sensitive individuals was 7/10 in offspring of DN+ vs. 2/10 in offspring of DN-. In the groups high salt diet caused a comparable decrease of PRA and aldosterone accompanied by an increase in ANF.
The results indicate that blood pressure is more salt sensitive in offspring of type 2 diabetic parents with as opposed to offspring of parents without diabetic nephropathy. Salt sensitivity of blood pressure apears to be an intermediate phenotype linked to, or perhaps pathogenetically involved in, the genesis of diabetic nephropathy.

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ACE-INHIBITOR-INDUCED COUGH IN TYPE 2 DIABETIC HYPERTENSIVES

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Aim: ACE-Inhibitors (ACE-I) represent first line drugs in diabetic pts in whom, however, the incidence of drug-induced dry cough was never specifically investigated. Thus we were prompted to carry out the present large survey. Materials and Methods: During a four-month period (Apr-Jul 1998), all type II diabetic pts consulting at the Diabetes Clinic of our Hospital were interviewed by physician-led questionnaire to assess whether they were hypertensive and, if so, the type of pharmacologic regimen taken, and to verify the appearance while on treatment of dry cough and of seven other proposed confounding side-effects. Pts were unaware that the questionnaire was focused on cough : when the symptom was declared, the cause-effect relationship to ACE-I treatment was determined. Results: We interviewed 2074 consecutive pts; 52% of them were treated pharmacologically for hypertension; 64% of treated subjects received an ACE-I (n= 691, m = 264; f = 427; mean age = 69±9 y; diabetes duration = 13±10 y). ACE-I-induced persistent, dry cough was detected in 14.9% (C.I. 12.2, 17.5). The symptom prevalence was not different in women (16.2%) and in men (12.9%), p=ns. Development of the side-effect had no relationship to age, diabetes duration, smoking status or type of hypoglycaemic treatment. Non-specific coughing was present in 4 1% of non-ACE-I treated pts. "Tickling in the throat" was declared by 38.2% of ACE-I coughers, by 7.9% of non-coughing ACE-I treated pts, and by 4.3% of non-ACE-I treated pts. Dry cough led to interruption of treatment in 4.7% of ACE-I treated pts, disappearing thereafter. Conclusions: The prevalence of the side-effect found in diabetic pts is twice the 7 4% ($p<0.05$) we previously observed in a similar survey in non-diabetic hypertensives Diabetes appears to increase the overall prevalence and to minimize the known gender related difference in ACE-I -induced cough.

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NEWLY ESTABLISHED TYPE-1 DIABETIC RATS FOR THE STUDY OF DIABETIC HYPERTENSION AND NEPHROPATHY

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Aim: Diabetic BB/OK rat does not develop diabetic complications like hypertension, nephropathy or myocardiopathy. Therefore, we established several congenic BB rat strains transferring chromosomal regions with blood pressure QTL of the spontaneously hypertensive rat (SHR) onto the genetic background of BB/OK rats. However, the mean blood pressure effect measured in these congenic rat strains was small (+8-10 mmHg). That prompted us to chose the classical procedure of phenotypic selection to fix major genes of hypertension in the BB/OK rat subline. **Materials and Methods:** Diabetic BB/OK rats were crossed with hypertensive SHR/Mol rat. The F1 hybrids were hypertensive (196 ± 10 mmHg) and were backcrossed onto diabetic BB/OK rats resulting first backcross hybrids (BC1). BC1 hybrids with the highest blood pressure were backcrossed onto diabetic BB/OK rats. This procedure was repeated 7 times (N8). Animals of the 7th backcross generation (BC7) were phenotypically and genetically analysed using 240 microsatellite markers to select those rats with highest blood pressure and homozygosity for BB rat alleles to establish a hypertensive and diabetic BB.SHR congenic inbred subline. **Results:** 90% of BC7 rats developed diabetes and were characterised by a mean blood pressure of 182 ± 11 mmHg. Comparing the 24h excretion of urine constituents in diabetic BB/OK males (n=10) with diabetic BC7 males (n= 8) 3 weeks after diabetes onset, a significantly increased excretion of albumin (30 ± 19 vs. 78 ± 28 µg/24h, $p<.001$) and total protein (28 ± 11 vs. 48 ± 15 mmol/24h, $p<.005$) were registered. The genetic analysis indicated that the BC7 animals were already homozygous for the BB/OK rat alleles at all loci tested. **Conclusion:** With the aid of phenotypic selection it was possible to create a congenic BB.SHR subline developing most probably not only hypertension comparable with those of SHR (182 ± 11 vs. 197± 15 mmHg) but also nephropathy indicated by proteinuria 3 weeks after diabetes onset. Therefore, major genes of SHR affecting blood pressure regulation must have been transferred. Genetic fine mapping will help to identify the transferred major genes. A work going on.

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Lipids – General

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SERUM LIPID VALUES AND CENTRAL OBESITY IN TYPE 1 DIABETES - THE EURODIAB IDDM COMPLICATIONS STUDY

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There is evidence that increase of abdominal fat is associated with insulin resistance and cluster of coronary risk factors, such as dyslipidemia, arterial hypertension and increased thrombogenicity. **The aim** of this study was to assess the relationship between serum lipids and abdominal obesity assessed as waist to hip (W/H) ratio in 3250 type 1 diabetic patients attending 31 clinics in 16 European countries. **Material** included 1629 men and 1530 women with type 1diabetes, aged [mean, (SD)] 32,5 (10.2) and 32.5 (10.1) yrs, with diabetes duration 14.3(9.3) and 14.8 (9.3) yrs, and with HbA1c 6.7(1.8)% and 6.8(1.9)% respectively. **Methods:** All procedures were performed using standardized methods. Blood lipids were measured centrally, by enzymatic methods. All serum lipid values were significantly associated with age, HbA1c and albumin excretion rate (AER) and therefore all analyses has been age, HbA1c and AER adjusted. **Results:** Total cholesterol (TC), LDL-C ($p<0.002$), TC/HDL-C and fasting triglycerides (TG) ($p<0.0001$) increased and HDL-C ($p.<0,001$) and HDL3-C ($p<0.0001$) decreased with increase of W/H category in males (p. values testing for trend). In females TG levels ($p<0.0003$) and TC/HDL-C ($p<0.0001$) increased and HDL-C ($p.<0.003$), HDL2-C ($p.<0.04$) and HDL3-C ($p.<0.03$) decreased with increasing W/H ratio. Multivariate analysis for plasma lipids, with demographic and biochemical risk factors as the covariates, and in addition age, HbA1c and AER, revealed, that W/H ratio was significantly related [regression coefficients (95%CI)]: to HDL-C -.0.03 (-0.05, -0.01) $p<0.01$; HDL3-C -0.02 (-0.03, -0.01), $p<0.01$; and TC/HDL-C 0.03 (0.01, 0.05), $p<0.001$ in males; while to: TC 0.06 (0.01,0.11), $p<0.05$; TC/HDL-C 0.02 (0.01, 0.04), $p<0.01$, and TG 0.03 (0.01, 0.06), $p<0.05$ in females. **Conclusions:** The results indicate, that serum lipid levels are significantly associated with W/H ratio in IDDM patients, especially in men, and this may be of importance in obtaining goal lipid levels in this population with high risk of cardiovascular diseases.

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COMPOSITION AND OXIDATION POTENTIAL OF VERY LOW DENSITY LIPOPROTEIN SUBFRACTIONS IN TYPE 2 DIABETES

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In type 2 diabetes VLDL is enriched with triglyceride. We have developed a rapid ultracentrifugation method for separating 4 subfractions of VLDL A→D; (A is the largest least dense subfraction). Aims: To determine if the lipid content of VLDL subfractions and their susceptibility to copper induced oxidation are altered in patients with type 2 diabetes. Materials and Methods: 15 patients with type 2 diabetes (HbA1c 6.9%) were compared with 15 age and sex matched controls. Each subfraction was assayed for triglyceride, cholesterol, fatty acid, apo B and preformed hydroperoxide (HPO) content. Lag time to initiation of conjugated diene production by copper was used as a marker of susceptibility to oxidation. Results: Patient triglyceride standardised for apo B was higher in subfractions A, B & C than controls: A, 192* vs 46; B, 128* vs 30; C, 57* vs 13, Sub D 9 vs 5 (µmol trig/mg apo B; *$p<0.05$). MUFAs, expressed as percent of total fatty acid were lower in all 4 patient subfractions: A, 35* vs 37; B, 35* vs 39; C, 34* vs 37; D, 33* vs 36 (%; $p<0.05$). HPOs standardised for apo B content were higher in the patient group than controls: A, 340* vs 48; B, 346* vs 42; C, 262* vs 28; D, 54* vs 16 (nmol HPO/mg apo B; $p<0.001$). Lag time results indicate that as particles decrease in size (A→D) they become more susceptible to oxidation in both groups. Direct comparison of patient and control lag time indicates that patient subfractions A & D were more susceptible to oxidation compared to controls: A, 121* vs 137; B, 104 vs 113; C, 94 vs 104; D, 62* vs 79 ($p<0.05$). Conclusions: These results reflect abnormalities in VLDL subfraction composition and oxidation profile in well controlled type 2 diabetes these may contribute to the development of cardiovascular disease.

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RELATIONSHIP BETWEEN LIPID-TOLERANCE AND GLUCOSE-INTOLERANCE IN DIFFERENT STAGES OF GLUCOSE TOLERANCE.

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Aims: Abnormalities in postprandial (pp) clearance of triglyceride rich lipoproteins in subjects with type 2 diabetes are suggested to play an important role in atherogenesis, and have been observed by several investigators. So far, little information exists on pp changes in TG-rich lipoproteins in subjects with IGT. Therefore, we analysed pp TG and lipoprotein concentration in relation to glucose tolerance. **Materials and methods:** An oral fat-glucose meal (93g fat, 82g glucose) was administered in age- and body mass index matched men with impaired glucose tolerance (IGT; n>11), in patients with type 2 diabetes (n>11) and control subjects with normal glucose tolerance (NGT; n=20). All individuals examined were nonsmokers. Plasma lipoprotein levels were analysed in a fasting state as well as every hour within 6 hours after the load. All subjects had fasting triglycerides < 2,3mmol/l. **Results**: The pp hypertriglyceridaemia by calculation of the „areas under the curves" (AUC) was greater in patients with type 2 diabetes compared with IGT ($p<0,05$) and control group ($p<0,05$). No significant correlation of TG AUC was found in IGT (10,5 mmol/lx6h) group compared with control group (11,8mmol/lx6h). Fasting triglyceride concentration in VLDL 1 fraction (S_f 60-400) and in VLDL 2 fraction (S_f 20-60) and pp TG concentration in VLDL1 were significantly higher in diabetes than in IGT and controls, but not in IGT group compared with control group. AUC of TG significantly correlated to AUC of plasma glucose ($p<0,01$) and AUC of insulin ($p<0,05$). **Conclusion**: Subjects with IGT did not exhibit differences in pp plasma TG responses and TG level in the VLDL 1, VLDL 2. However in the whole spectrum glucose tolerance and pp insulin were significant determinants of lipid tolerance. Our data show that type 2 diabetes and not IGT is associated with lipid intolerance.

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Title: Leptin is not related to postprandial triglycerides in type 2 diabetes mellitus.

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Aim: Postprandial lipemia is an independent risk factor for conorary heart disease in type 2 diabetes mellitus. Our study investigates the relation of fasting plasma leptin levels to postprandial triglycerides in diabetic patients after an oral fat load test.

Methods: The study group consisted of 39 type 2 diabetic patients (34 males and 5 females). BMI,WHR and blood pressure was determined to all the participants. After an overnight fast, blood samples were drown for the determination of leptin, insulin and proinsulin (RIA). Patients consumed a fatty meal of 677 Kcal/m^2 consisting of 82%fat, 3.2% protein and 14.8% carbohydrates, with 300,000 IU of vitamin A added. Blood samples for the determination triglycerides (Trg) (enzymatic method) were drawn at baseline and 2,4,6 and 8 h after the fatty meal. The area under the curve (Trg AUC) was calculated by plotting the concentration of trg over the 8 h, using the 'trapezoidal rule'. Patients with hepatic, thyroid and renal dysfunction and patients under hypolipidemic therapy were excluded from the study. Statistical analysis was performed using Pearson correlation coefficient and linear regression analysis. The level of significance was at $p<0.05$.

Results: Postprandial triglycerides, expressed as total Trg AUC, significantly correlated with fasting Trg ($r=0.887,p=0.0001$), WHR ($r=0.34,p=0.34$) , fasting insulin ($r=0.489,p=0.082$) and proinsulin ($r=0.384$, $p=0.016$). On the contrary, BMI ($r=0.247,p=0.13$) and leptin ($r=0.025,p=0.882$) did not show statistically significant correlation's with postprandial triglyceride levels. Using linear regression analysis, leptin did not have independent correlation to AUC TRG. On the contrary, fasting trg had independent corrrelation with postprandial triglycerides (adj $R^2=0.597$,beta=0.89,$p=0.0001$).

Conclusion: Plasma leptin levels are not an independent predictor of postprandial triglyceridaemia in type 2 diabetes mellitus.

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ANALYSES OF CARDIOVASCULAR RISK FACTORS IN OFFSPRINGS OF TYPE 2 DIABETIC PATIENTS

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Aims: To evaluate the status of cardiovascular risk factors in offsprings of type 2 diabetic patients in comparison with subjects without a family history of type 2 diabetes. **Materials and Methods:** Systolic and diastolic blood pressure, serum total cholesterol, HDL, LDL and VLDL levels, serum fibrinogen concentrations and urinary albumin excretion rates were evaluated in 114 subjects with parental history of diabetes and 42 subjects without family history of diabetes. The median values of the variables were compared with Mann-Whitney U test and Spearman's correlation coefficients were calculated to assess the relations of these parameters with age, determinants of obesity (body mass index, waist to hip ratio), adiposity (biceps, triceps and subscapular skin thickness), fasting plasma glucose and fasting plasma insulin levels. **Results:** The two groups were comparable with respect to age, sex, body mass index, fasting plasma glucose and insulin levels, HDL-cholesterol, triglycerides, fibrinogen and urinary albumin excretion. The offsprings of diabetic patients had higher median systolic (110 vs. 100 mm-Hg, $p<0.05$) and diastolic (90 vs. 85 mm-Hg, $p<0.05$) blood pressure, median LDL-cholesterol (115 mg/dl vs. 85 mg/dl, $p<0.01$) and median total cholesterol (189 mg/dl vs. 150 mg/dl, $p<0.0001$) levels compared with controls. In the diabetic patients' offsprings group, total cholesterol levels correlated with systolic blood pressure ($r=0.28$, $p<0.05$), biceps ($r=0.32$, $p<0.05$) and subscapular ($r=0.26$, $p<0.05$) skin thickness. In this group, LDL-cholesterol correlated with biceps skin thickness ($r=0.36$, $p<0.0001$). None of the assessed risk factors showed any significant correlation with age, body mass index, waist to hip ratio, fasting plasma glucose and insulin levels. **Conclusions:** The data suggest that the relatively higher blood pressure, total cholesterol and LDL-cholesterol levels which are related with the degree of adiposity in the offsprings of diabetic patients might indicate to an increased cardiovascular risk in this population.

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DIFFERENCES IN APO E PHENOTYPE AND HDL-CHOLESTEROL: APO A-I +APO A-II RATIO WITH ALBUMINURIC STATUS IN TYPE 1 DIABETIC PATIENTS

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Aims: To examine whether apolipoprotein E phenotype and serum HDL- cholesterol: ApoA-I+ApoA-II ratio, are associated with albumin excretion rate (AER) in type 1 diabetic patients. **Methods:** The above parameters were measured in 617 patients aged 15-60 years from 7 European diabetic centres. AER, measured in a central laboratory, was defined as normoalbuminuria if ≤ 20μg/min, microalbuminuria 20-200μg/min, and macroalbuminuria ≥ 200μg/min. ApoA-I and ApoA-II concentrations were measured by an immunoturbidometric method (mmol/L). Apo E phenotype was measured using a rapid micromethod. **Results:** Among those with ≥1 ϵ2 allele (n=92) 34% had albuminuria (microalbuminuria 24%, macroalbuminuria 10%) compared with 29% in ϵ2-ve patients (p for trend =0.4). After adjustment for age, diabetes duration, HbA_{1c}, systolic blood pressure (SBP), LDL cholesterol, and fasting triglycerides the ϵ2 allele was associated with an odds ratio of 1.6 for albuminuria (p=0.2). The HDL-cholesterol: ApoA-I+ApoA-II ratio was inversely related with albuminuria being 0.89, 0.86 and 0.80 in those with normo-, micro- and macroalbuminuria respectively (p=0.001, adjusted for age and sex). However with further adjustment for diabetes duration, HbA_{1c}, BMI, SBP, LDL-cholesterol and smoking, this was not significant (p=0.2). **Conclusion:** In this study the association of ϵ2 allele with nephropathy, found in some previous studies in type 1 diabetes, was not replicated. We have found an inverse relationship of HDL-cholesterol: Apo A-I+Apo A-II ratio with AER in univariate analysis. However this relationship was not independent of the other risk factors associated with albuminuria.

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APOLIPOPROTEIN B-DEPENDENT DYSLIPIDAEMIC PHENOTYPES IN NORMOCHOLESTEROLAEMIC IDDM PATIENTS.

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We recently described an increased prevalence of apolipoprotein B (apoB)-dependent dyslipidaemic phenotypes in normocholesterolaemic type 2 diabetic patients. These phenotypes are associated with cardiovascular risk, and are not detected in routine lipidic evaluation. **AIM:** We studied the prevalence of dyslipidaemic phenotypes, including those dependent on apoB, in 50 normocholesterolaemic IDDM patients (40% males, aged 37.7 ± 13.7 years, BMI 24.7 ± 2.8 Kg/m^2, diabetes duration 12.9 ± 8.5 years, HbA1c 7.98 ± 1.7%) and 53 non-diabetic normolipidaemic (normocholesterolaemic and normotriglyceridaemic) subjects (39.6% males, aged 37.6 ± 9.4 years, 24.2 ± 3.3 Kg/m^2). **MATERIAL AND METHODS:** After an overnight fast, we measured: triglyceride (Tg) and total cholesterol (Tc) (automatic enzimatic methods), HDL cholesterol (direct method without precipitation) and LDL cholesterol (Friedewald's formula/ ultracentrifugation) and apolipoprotein B (immunoturbidimetry). Dyslipidaemia was defined as LDLc > 4.13 mmol/l, Tg > 2.25 mmol/l, and HDLc < 0.9 (males) or 1.17 mmol/l (females). The cut-off point for apoB (1.14g/l) was obtained from the non-diabetic normolipidaemic group. **RESULTS:** Seventy-six percent of the normo-cholesterolaemic IDDM patients were classified as normolipidaemic, 16% had low HDLc, 12% hyperapoB and 8% were hypertriglyceridaemic (vs 18.9% low HDLc and 9.4% hyperapoB in the control group; p: ns). HyperapoB was present in 2 of the 4 hypertriglyceridaemic, and in 4 of the 46 normotriglyceridaemic patients. Three of the 6 subjects with hyperapoB showed low HDLc. **CONCLUSION:** unlike NIDDM, IDDM does not seem to be associated with increased prevalence of apoB-dependent dyslipidaemic phenotypes.

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LEVELS OF AUTOANTIBODIES AGAINST OXIDIZED LDL IN DIABETIC SUBJECTS ARE HIGHER WHEN MACROANGIOPATHY IS PRESENT.

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Aim: Oxidation of LDL provokes the generation of antigenic epitopes which cause immune reactions. Autoantibodies against these epitopes (oLAB : oxLDL AutoantiBodies) can now be detected and they could represent a marker of lipid peroxidation. Aim of this study was to evaluate oLAB serum levels in diabetic patients and their correlation with the presence of macroangiopathy. Material and Methods: oLAB values were measured (ELISA BIOMEDICA) in 68 diabetic subjects (age 66±11, 48 females-20 males) and they were found significantly higher as compared to those of 25 normal sex and age-matched controls (436±291 mu/ml vs 189±64 mu/ml, p=0.003) .Clinical signs and history of overt macrovascular disease were recorded. Results: Regression analysis between oLAB as dependent variable and lipidaemic profile (cholesterol, HDL, LDL, triglycerides, ApoA1, ApoB, Lpa), serum urea and creatinine, BMI, WHR, blood pressure values, HbA1c , age and the presence of macroangiopathy showed a positive correlation between oLAB and the presence of macroangiopathy (p=0.002).Diabetic subjects with macroangiopathy presented higher levels of oLAB as compared with diabetics without macroangiopathy(578±370 mu/ml [n=20]vs 378±225 mu/ml [n=48] , p=0.009) .Conclusions : a) oLAB levels are increased in diabetic subjects b) The levels are significantly higher in macroangiopathic patients. It is possible that increased LDL oxidation could be correlated with the presence and possibly the progression of macrovascular complication.

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SMOKING WORSENS INTERPRANDIAL LIPID PROFILE IN SUBJECTS WITH TYPE 2 DIABETES MELLITUS.

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Interprandial (IP) lipidaemia accounts for the bulk of nycthemeral exposure, and although smoking was reported to worsen fasting (F) lipids profile, its effect on IP lipids is poorly documented. **Aims**: To evaluate diabetic subjects in terms of IP and F lipids and smoking status. **Subjects**: 518 subjects with type 1 (T_1, n=191) or type 2 (T_2, n=327) diabetes mellitus (DM), either current smokers (CSm; n=48 (T_1) and 50 (T_2)), non-smokers (NSm: n=110 (T_1) and 178 (T_2)) or past-smokers (PSm: n=33 (T_1) and 99 (T_2)). **Results**: More males were smokers or past-smokers (%): 69 (T_1CSm), 79 (T_1PSm), 82 (T_2CSm) and 88 (T_2PSm) *vs.* 39 in T_1 or T_2NSm (p<0.01). HbA_{1c} was highest in T_1CSm (8.84±1.01 *vs.* 8.33±1.33% in T_1NSm; p<0.02) but not different between T_2 subjects (≈8.30%). Daily insulin dose was not different between T_1NSm and CSm, nor was BMI between T_1NSm and CSm, nor between T_2NSm and CSm. T_1CSm had more retinopathy, neuropathy and micro-or macroalbuminuria than T_1NSm (p<0.02), while T_2CSm and T_2PSm had respectively more peripheral and coronary artery diseases than T_2NSm (p<0.05). Hypolipemic drug use was alike between T_1 or T_2CSm and NSm. T_1NSm and T_1CSm had similar fasting triglycerides (TG_F), total cholesterol (TC_F), LDL-C_F, and interprandial TG (TG_{IP}), TC_{IP}, LDL-C_{IP} or TC.HDL-C^{-1}_{IP}, although HDL-C_F and TC.HDL-C^{-1}_F were different in CSm (55±17 *vs.* 67±28 mg.dL^{-1} and 3.8±1.3 *vs.* 3.2±1.0 in T_1NSm, p<0.01). In T_2 subjects, TG_F, TC_F and TC.HDL-C^{-1}_F were not different between NSm and CSm, while HDL-C_F was lower in CSm (40±9 *vs.* 47±14 mg.dL^{-1} in NSm, p<0.001). By contrast, there were marked differences in IP lipids between CSm and NSm with T_2 DM. Thus, in T_2CSm, TG_{IP} were 207 [128-368] *vs.* 162 [115-245] (median [perc25-75]; p<0.05), LDL-C_{IP} 143±41 *vs.* 127±32 (p<0.02), HDL-C_{IP} 43±15 *vs.* 50±17 mg.dL^{-1} (p<0.01), and TC HDL-C^{-1}_{IP} was 6.0±2.2 *vs.* 4.7±1.8 (p<0.001). These abnormalities were not found in T_2PSm. **Conclusions**: In type 2 diabetes, smoking markedly affects interprandial lipids. These specific changes are of atherogenic character, and were not found in past-smokers with type 2 diabetes, nor in smokers with type 1 diabetes.

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Lipids – Interventions

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EFFECTS OF ATORVASTATIN ON PLASMA HOMOCYSTEINE LEVELS IN SUBJECTS WITH FAMILIAL HYPERCOLESTEROLEMIA.
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Aims: Mildly elevated total homocysteine levels confer an indipendent risk of vascular disease. Adverse vascular effects of homocysteine involve endothelial injury and smooth-muscle cells proliferation besides oxidation of low-density lipoprotein (LDL). A positive correlation between plasma levels of homocysteine and LDL-ch has been recently described. **Materials and Methods**: To evaluate the effects of atorvastatin on plasma homocysteine levels, 22 subjects with primary hypercholesterolemia (14 males, 8 females) discontinued their lipid lowering therapy for at least six weeks. All patients were non-diabetic (OGTT), non-obese (25.0±3.0 kg/m^2) and normotensive (129/81±11/7 mmHg, ambulatory blood pressure monitoring). Lipids (enzymatic colorimetric techniques), lipoproteins (nephelometry) and homocysteine (Axis Biochemicals ASA, Oslo, Norway) were measured at baseline, one-month (atorvastatin 20 mg per day in a single nocturnal dose) and six-month (40 mg per day). Twenty-two well-matched healthy subjects acted as controls. **Results:** Baseline homocysteine levels were higher in hypercholesterolemic patients than in controls (13.0±3.6 vs 10.1±3.9 µmol/l, p=0.013). ANOVA for repeated measures was employed to evaluate the effects of atorvastatin in hypercholesterolemic patients. Atorvastatin reduced LDL-ch by about 45% (from 297±47 to 157±36 mg/dl) with most lowering at one-month, 20 mg treatment (180±37 mg/dl), (p=0.0001). Homocysteine levels did not change at one-month, 20 mg treatment (13.1±3.4 µmol/l), but was significantly reduced at six-month, 40 mg treatment (10.9±2.9 µmol/l; p-value between subjects=0.0003, between treatments=0.0059). Percent change in homocysteine levels was related to baseline homocysteine values (r=0.65, p=0.001), but not to percent reduction in LDL-ch (r=0.21, p=0.34). **Conclusions:** Independently of lowering of LDL-ch, atorvastatin treatment reduces total homocysteine levels in primary hypercholesterolemic patients. Both molecular mechanisms of homocysteine reduction and its putative role in enhancing desirable effects of statins are unknown at the present time. Improvement of endothelial dysfunction may be assumed as a hypothesis.

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EFFECT OF FLUVASTATIN TREATMENT ON RED BLOOD CELL CATION TRANSPORT
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Hyperlipidemia frequently observed in patients with diabetes mellitus may alter membrane lipid composition and affect membrane cation transport activities. The **aim** of the study was to evaluate the effect of a 3-week treatment with fluvastatin on erythrocyte sodium transport. **Methods:** We measured the activities of Na^+-K^+ pump, Na^+-K^+ cotransport, passive Na^+ permeability and Na^+-Li^+ countertransport in 20 patients with diabetes mellitus type 2 treated with glibenclamide. After a 3-week run-in period the patients were randomized into two groups, receiving either fluvastatin (n=12; F) or placebo (n=8; P). Placebo or fluvastatin (total dose of 80 mg) were administered twice a day. **Results:** Despite no effect on intracellular sodium concentrations (F: 6.87 ± 1.58 v.s. 6.83 ± 1.84 mmol.l^{-1}.h^{-1}; P: 6.02 ± 1.10 v.s. 5.93 ± 0.94 mmol.l^{-1}h^{-1}), fluvastatin decreased the activity of the erythrocyte Na^+-Li^+ countertransport compared to placebo (F: 0.87 ± 0.41 v.s. 0.66 ± 0.31 mmol.l^{-1}.h^{-1}; p<0.05; P: 0.48 ± 0.33 v.s. 0.65 ± 0.21 mmol.l^{-1}.h^{-1}). No changes in Na^+-K^+ pump (F: 7.94 ± 2.71 v.s. 7.69 ± 2.45 mmol.l^{-1}.h^{-1}; P: 6.57 ± 3.54 v.s. 6.66 ± 2.32 mmol.l^{-1}.h^{-1}), Na^+- K^+ cotransport (F: 0.73 ± 1.00 v.s. 0.81± 0.59 mmol.l^{-1}.h^{-1}; P: 0.55 ± 0.41 v.s. 0.60 ± 0.26 mmol.l^{-1}.h^{-1}), and passive Na^+ permeability (F: 0.114 ± 0.046 v.s. 0.106 ± 0.026 mmol.l^{-1}.h^{-1}; P: 0.127 ± 0.10 v.s. 0.105 ± 0.03 mmol.l^{-1}.h^{-1}) were found. **Conclusions:** The data show that cholesterol lowering treatment with fluvastatin in type 2 diabetic patients is accompanied by a decrease in Na^+-Li^+ countertransport activity in red blood cells.

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CHANGES OF SERUM STEROID HORMONE LEVELS IN PATIENTS WITH TYPE 2 DIABETES AFTER TREATMENT WITH CERIVASTATIN
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Aims: Cerivastatin reduces the adrenal content of cholesterol in rats, however, little is known about the influence of cholesterol-lowering therapy on steroid hormone levels in men. We analysed the effect of cerivastatin treatment on steroid hormones levels in patients with type 2 diabetes. **Methods:** 31 patients (12 female, 19 male) were randomly divided into two groups. Group 1 (n=15) received 0.1 mg Cerivastatin and group 2 (n=16) 0.3 mg. Serum levels of lipids, cortisol, estradiol (E2) and testosterone were measured in 31 patients before therapy, as well as after 1, 2, 3 and 4 months of treatment. Statistics were calculated utilizing the Wilcoxon test. **Results:** A significant reduction of total serum cholesterol was found only after 4 weeks of treatment (Group 1: 5.6%, p=0.0012; group 2: 26.2%, p=0.0003). LDL-cholesterol also decreased (group 1: 47.3%, p=0.001; group 2: 55.8%, p=0.0007). A decrease in serum triglycerides was only significant in group 2 (Group 2: 28%, p=0.0016). Changes of sex hormone levels were only found in female patients. A decrease of E2 (from 16.919 ± 3.32 to 12.488 ± 5.25, p=0.35, all values mean ± SE) and testosterone (from 52.62 ± 39.23 to 35.5 ± 13.76, p=0.035) was displayed (only group 2). Furthermore, after 4 months of anti-cholesterol therapy, basal serum cortisol levels were lower compared to baseline levels (Group 2: from 14.38 ± 2.8 to 12.05 ± 2.21, p=0.04). **Conclusion:** Cerivastatin affects steroid hormone levels in female patients in a dose-dependent manner. In male patients, no change was found in total circulating testosterone or estradiol. In women, testosterone is mainly bound to SHBG (66%, men: 44.3%). Further studies are necessary to elucidate wether bioactive steroid hormone levels or transport proteins are influenced by anti- cholesterol therapy.

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HYPOTENSIVE EFFECTS OF STATINS: PRELIMINARY DATA WITH ATORVASTATIN.
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Aims: Endothelium-dependent vasodilation is abnormal in patients with athero-sclerosis and hypercholesterolemia. Lipid-lowering therapy with statins restores or improves endothelial dysfunction. Objectives of our study were to evaluate the effect of atorvastatin in reducing of total- and LDL-cholesterol and to monitor the putative properties in lowering of arterial blood pressure. **Materials and Methods:** Eighteen non-diabetic, non-obese, no smoking, normotensive subjects (11 males, 7 females; 13 with proven familial hypercholesterolemia and five with polygenic hypercholesterolemia; age 49.3±8.6 years, BMI 25.1±3.1 kg/m^2) discontinued their lipid-lowering drugs for at least six weeks. Therefore they started atorvastatin treatment at 20 mg per day for 1 month and then at 40 mg up to six months. Lipid profile (total- and LDL-cholesterol, triglycerides, ApoA1 and ApoB) and ambulatory blood pressure monitoring (SpaceLab 90207) have been measured at baseline, 1 month and six months. ANOVA for repeated measures (one trial factor) and, if differences were found, Scheffe's F-test, were used to evaluate the effects of atorvastatin. **Results:** Atorvastatin reduced LDL-cholesterol by about 45% from 297±47 to 157±36 mg/dl with most of the reduction occurring during the 1st month (180±37 mg/dl), (p=0.0001). Total-cholesterol, triglycerides and ApoB reduced by about 39%, (p=0.0001), 23% (p=0.0002) and 39% (p=0.0001) respectively. Systolic blood pressure reduced from 128±10 to 120±8 mmHg, with most of the reduction occurring at 1 month (121±12 mmHg) (p=0.0027). Diastolic blood pressure moved from 80±6, throught 75±8 (1 month) to 75±7 mmHg (p=0.0095). No significant regressions were found between percent reduction in LDL-cholesterol levels and percent reduction in both systolic (r=0.28, p=0.26) and diastolic BP (r=0.34, p=0.17). **Conclusions:** Atorvastatin administered at 20-40 mg per day to 18 normotensive patients lowers the cholesterol level and causes a prompt and sustained reduction in blood pressure. Lowering of blood pressure in independent of lowering of LDL-cholesterol.

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This abstract has been withdrawn

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VITAMIN E, FENOFIBRATE AND THEIR COMBINATION ON NON HDL LIPOPROTEIN OXIDABILITY IN PATIENTS WITH TYPE 1 DIABETES

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Oxidative stress and lipid peroxidation could contribute to the development of complications in type 1 diabetes mellitus (T_1DM). **Aims:** Following pilot studies which indicated that micronised fenofibrate alone or in combination with Vitamin E was able to prolong lag time of oxidation of atherogenic lipoproteins in T_1DM, we conducted a double-blind placebo-controlled parallel group trial in the same patient population. **Materials and Methods:** After a 2 month placebo run-in period (Period A) 44 T_1DM patients (27 men, 17 women) either dyslipidemic or not were randomly divided into 3 groups. Group 1 (10M, 4W) received 400 IU RRR α tocopherol acetate (Vit E) once daily (od) for 2 successive periods of 2 months each (Periods B and C). Group 2 (10M, 6W) received micronised fenofibrate 200mg od (F) for 2 months and then the combination of 400 IU RRR α tocopherol acetate and 200mg micronised fenofibrate od for 2 months. Group 3 (7M, 7W) continued placebo (P) for 4 months. At the end of each period, after precipitation of HDL, VLDL+LDL lipoprotein oxidability was measured ex vivo as lag time of the propagation phase of fluorescent conjugated dienes formation after addition of copper sulfate. **Results:** Following Period A, HbA_{1c} was 7.6%, glucose 9.7 mmol/l, total cholesterol (TC) 5.2 mmol/l, triglycerides (TG) 0.95 mmol/l, HDL cholesterol 1.53 mmol/l and the 3 groups did not differ. The study was completed by 42 patients without significant clinical/ biological adverse events. In the 3 groups, HbA_{1c} was unchanged. TC fell by 20% and TG by 24% in the Group 2 only. The combination F+Vit E led to a significant 17 min increase in VLDL+LDL oxidation lag time ($p<0.05$ vs Period A).

Lag time m(sd)	Period A	Period B	Period C
Vit E, min	120 (25)	124 (21)	121 (13)
F, F+Vit E	101 (25)	112 (23)	118 (22)
P	108 (24)	113 (23)	110 (25)

Conclusion: The synergism between Vitamin E and micronised fenofibrate on protection of non HDL lipoprotein oxidability ex vivo demonstrated in this randomized placebo-controlled study in T_1DM awaits confirmation in further studies.

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THE EFFECT OF AN OLEIC VS LINOLEIC ACID-ENRICHED DIET ON POST PRANDIAL APO B48 AND B100-CONTAINING LIPOPROTEINS IN DIABETIC AND CONTROL SUBJECTS.

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Post prandial lipoprotein remnants are thought to be particularly atherogenic. **Aim** To compare a mono- to a polyunsaturated fat diet on postprandial lipoproteins. **Materials and methods** 6 type 2 diabetic patients and 6 control subjects were examined in a random cross-over study. At the end of each dietary period (2 weeks) fasting, 2, 4, 6, and 8 hour samples were collected following an 1100 Kcal meal. Fasting insulin and glucose were significantly higher on the linoleic acid diet in the diabetic patients ($p<0.05$. **Results** Total cholesterol was significantly higher in both control and diabetic patients on the linoleic acid. Apo B48 and B100 were determined by gradient gel electrophoresis. On the linoleic acid diet diabetic patients had significantly more chylomicron apo B48 than control subjects (AUC 57.9±89 vs 12.1±2.7 $p<0.005$) and apo B100 (104.3±16.1 vs 22.9±5.9, 0.001) and also for VLDL apo B48 and B100 were AUC 28.0±8.7 vs 11.8±1.5 for apo B48 and 256.5±49.8 vs 123.5±30.3 $p<0.05$). On the oleic acid diet diabetic patients had more chylomicron apo B48 (27.8±2.5 vs 8.5±1.7, $p<0.0001$) and apo B100 (50.3±7.3 vs 21.1±4.6 $p<0.05$). VLDL apo B48 or B100 were not different. **Conclusions** Alteration of dietary fatty acids does not effect post prandial apo B48 or apo B100 in control subjects. In diabetes an oleic acid diet improves, but does not normalise, chylomicron apo B48 and B100 but normalises VLDL apo B48 and B100. These results may have implications in prevention of atherosclerosis.

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AT(1) RECEPTOR ANTAGONISM LOWERS PLASMA TOTAL AND VLDL+LDL CHOLESTEROL IN TYPE I DIABETIC PATIENTS WITH ALBUMINURIA.

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Aims: To determine the effects of AT1 receptor antagonism on the lipoprotein profile and on plasma cholesterol esterification and cholesteryl ester transfer in Type 1 (insulin-dependent) diabetic patients with low range albuminuria.

Methods: In a single blind longitudinal study 8 patients were studied at baseline, after 28 days of losartan treatment (50 mg daily), followed by a 28 days recovery period.

Results:

	baseline	losartan	recovery
albuminuria(mg/24h)	188 (47-1057)	94 (21-337)*	151 (43-878)
total chol (mmol/l)	5.25±0.28	4.91±0.25*	5.14±0.18
VLDL+LDL (mmol/l)	3.84±0.31	3.59±0.27*	3.79±0.22
HDL (mmol/l)	1.40±0.13	1.32±0.12	1.35±0.12
apo B (g/l)	0.74±0.06	0.68±0.06*	0.71±0.06
apo AI (g/l)	1.57±0.10	1.49±0.10*	1.54±0.09

Median (range) and mean ± sd. *:$p<0.05$ vs average value baseline and recovery.

Plasma cholesterol esterification and cholesteryl ester transfer were unchanged. Changes in plasma total and VLDL+LDL cholesterol were correlated with changes in albuminuria (R_s= 0.76, n=8, $p<0.05$ and R_s= 0.79, n=8, $p<0.05$, respectively).

Conclusions: In Type I diabetics with low range albuminuria, the AT1 receptor antagonist losartan lowers atherogenic plasma apo B-containing lipoproteins in conjunction with a decrease in albuminuria. The unchanged plasma cholesterol esterification and cholesteryl ester transfer indicate that these determinants of HDL metabolism remain unaffected.

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EFFECT OF GLYCEMIC CONTROL ON THE PREVALENCE OF DYSLIPIDAEMIA IN IDDM PATIENTS.

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Unlike NIDDM, data on the prevalence of dyslipidaemia in IDDM patients are scarce, and mostly based on total cholesterol (Tc) and triglyceride (Tg) in cross-sectional studies. **AIM:** To determine the effect of glycaemic control optimization on the prevalence of dyslipidaemia in non-ketotic IDDM patients with poor glycaemic control. **MATERIAL AND METHODS:** Three-hundred and thirty-four IDDM patients (54.2% male, aged 31.3 ± 10.2 years, BMI 22.6 ± 3.2 Kg/m^2, diabetes duration 7.9 ± 9.3 years, HbA1c 8.5 ± 2%) and 803 non-diabetic subjects (48.7 % male, aged 30.7 ± 9.2 years, BMI 24.8 ± 4.4 Kg/m^2) were studied. Assessment was performed on the patients and compared with the control group at entry and after achieving good glycaemic control (HbA1c 6.3 ± 1.3%) by means of intensive therapy. Tg and Tc (automatic enzymatic methods), HDLcholesterol (precipitation) and LDL cholesterol (Friedewald's formula/ ultracentrifugation) were determined after an overnight fast. The cut-off points for dyslipidaemia were defined as LDLc> 4.13 mmol/l, Tg > 2.25 mmol/l, HDLc <0.9 mmol/l for males and 1.1 mmol/l for females. **RESULTS:** Among the poorly-controlled IDDM patients, LDLc>4.13, Tg> 2.25 and low HDLc were found in 15%, 5% and 20%, and in 14%, 6% and 9% of controls, respectively ($p < 0.05$ for HDLc). After improvement of glycaemic control, the prevalence of LDLc >4.13, Tg > 2.25 and low HDLc decreased to 9, 3 and 11%, respectively. In this stage of glycaemic control, the frequency of dyslipidaemia was not different between IDDM women and their controls. IDDM males, however, displayed less hypercholesterolaemia than non-diabetic men (8.3 vs 17% $p < 0.01$). **CONCLUSION**: The prevalence of low HDLc, but not hypercholesterolaemia or hypertriglyceridaemia is increased in poorly controlled IDDM patients. After achieving good glycaemic control, the prevalence of dyslipidaemia is similar or less than in the non-diabetic population.

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STRUCTURED GROUP EDUCATION ON HYPERLIPIDAEMIA IN DIABETES: BENEFICIAL LONG-TERM EFFECTS

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Pharmacotherapeutic interventions in hyperlipidaemia have already been evaluated according to the principles of evidence-based medicine. It has already been proven that structured education can enhance life style modifications and increase compliance with treatment.

Aim: To develop a structured outpatient group education model for hyperlipidaemic patients with type 2 diabetes and/or metabolic syndrome, not sufficiently responding to individual counselling and to evaluate long-term effects of such intervention. Main outcome criteria: cardiovascular risk profile, cholesterol/HDL ratio, compliance with pharmacotherapy.

Methods: Outpatient structured group education consisting of two 3-hours units. Contents: Atherosclerosis risk factors, causes and consequences; Pathophysiology of lipid metabolism; Blood lipid target values; Classification of hyperlipidaemia; Nonpharmacological therapy and nutrition change; Pharmacotherapeutic agents and their indications: fibrates, statins etc.; Interpretation of package information leaflet; Supervised self-classification of the degree of hyperlipidaemia according to individual results of blood analysis including individual therapy measures.

Results: The educational model was applied in 79 patients (diabetes type 1 n=48%, type 2 n=40%, no diabetes n=12%; observation time 52±20 months) with following treatment effects: cholesterol -0.3 mmol/l, p=0.18; HDL-cholesterol +0.6 mmol/l, p<0.001; LDL-cholesterol -0.02 mmol/l, p=0.5; cholesterol/HDL -1.0, p<0.001; triglycerides -2.6 mmol/l, p=0.06. The pharmacotherapy of hyperlipidamica was necessary in long-term in 52% of patients and was kept to in the majority of these patients.

Conclusions: The developed structured hyperlipidaemia group education model enables an effective improvement of cardiovascular risk profile.

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Neuropathy – General

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PERIPHERAL NERVE FUNCTIONS IN NEWLY DIAGNOSED SEVERELY HYPERGLYCEMIC DIABETIC PATIENTS

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Aims: Taking advantage of a unique group of young diabetic patients in Bangladesh who are severely hyperglycemic (fasting blood glucose usually >16 mmol/l), and normo- to hypoinsulinemic without being ketotic we have studied a group of newly diagnosed, neurologically asymptomatic subjects to explore the effect of relatively acute severe hyperglycemia on peripheral nerve functions and to assess whether motor or sensory functions are affected first. **Subjects and Methods:** Thirty diabetic patients (Age in years; 24.09±3.94 and BMI, 19.13±3.16, M±SD) were studied along with 30 control subjects having no family history of diabetes (Age in years; 20.83±3.15 and BMI, 19.35±2020, M±SD). Apart from blood glucose (glucose-oxidase method) and serum fructosamine (colorimetric method) platelet aggregation were also measured optically to investigate its role in the early pathological changes. Nerve functions, as detected by EMG, were evaluated by determination of distal latency (DL), compound muscle action potential (CMAP), F wave latency (FWL) and motor nerve conduction velocity (MNCV) of *n. ulnaris* and *n. peroneus* and DL, sensory nerve action potential (SNAP) and sensory nerve conduction velocity (SNCV) of *n. ulnaris* and *n. suralis*. **Results:** The diabetic patients were severely hyperglycemic (Blood glucose, mmol/l was 18.65±6.73 and serum fructosamine value 644.01±226.01, M±SD). Significant differences were observed between diabetic vs control groups regarding MUCMAP, μv (M±SD; 4.78±1.60 vs 6.11±2.08; p<0.001) MUFWL, ms (31.14±13.61 vs 24.86±1.85; p<0.01), MUNCV, m/s (57.82±6.85 vs 66.48±6.29; p<0.001), MPCMAP, μv (5.29±2.48 vs 9.08±3.55; p<0.001), MPNCV, m/s (41.65±7.34 vs 52.60±8.74; p<0.001) and SSNCV, m/s (34.58±14.27 vs 42.38±8.52; p<0.01). A highly significant negative correlation was seen between the latency period and NCV. In diabetic patients blood glucose and fructosamine levels showed strong correlation with MPNCV (Glucose, -0.47/0.01 and fructosamine, -0.58/0.001; r/p) and MUNCV (Glucose, -0.41/0.05 and fructosamine, -0.47/0.00; r/p). Platelet aggregation was higher only in relation to motor nerves. **Conclusions:** Relative short- time exposure to severe hyperglycemia seems to be an independent factor for the development of neuropathy in diabetic patients. In early stage motor nerve functions are more widely affected and it may have a link with platelet hyperaggregation.

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PHRENIC NERVE FUNCTION IN TYPE 1 DIABETES

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Introduction : In type 1 diabetes, peripheral neuropathy (PN) is common both as a clinical complication and as a neurophysiological finding, while little is known about the occurrence of a neuropathy of the phrenic nerve combined with peripheral nerve involvement. Data on this issue are still conflicting and not easily comparable. **Aims** : To compare the phrenic nerve (right and left) function, as assessed by non-invasive neurophysiological tests, in two groups of type 1 diabetic patients, one without and one with PN. **Materials and Methods** : 34 type 1 diabetic patients : 17 without (PN–) and 17 with (PN+) electrodiagnostic signs of PN (mean ± SD; age 38.4 ± 9.4 vs 45.6 ± 10.5 yr; diabetes duration 16.6 ± 8.2 vs 26.2 ± 9.8 yr, respectively). Thirtyfour healthy subjects served as controls. The mean HbA1c concentration, used as index of the diabetic control in the 3 years preceding the study, was lower in the PN– as compared to the PN+ (7.2 ± 1.8 vs. 8.9 ± 2.0 %; p<0.1). Creatinine levels were in the normal range and similar (p=NS) in both groups.

Results : There was no statistical difference between PN– and PN+ in phrenic nerve latencies (right : 8.9 ± 1.0 vs 8.9 ± 1.1 msec, p=NS; left : 8.6 ± 0.9 vs 8.9 ± 1.1 msec, p=NS, respectively) and amplitudes (right : 0.80 ± 0.30 vs 0.83 ± 0.31 mV, p=NS; left : 0.82 ± 0.29 vs 0.82 ± 0.30 mV, p=NS, respectively) . Moreover, phrenic nerve latencies and amplitudes in both groups were similar (p=NS) to the those of the control subjects. **Conclusions** : These data suggest that distal involvement of somatic peripheral nerves is not associated with more proximal disorders of the phrenic nerve function in type 1 diabetic patients.

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THE ASSOCIATION BETWEEN NEUROELECTROPHYSIOLOGY AND CLINICAL NEUROPATHY IN TYPE 1 DIABETES MELLITUS.

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Aims: This study examined the association between neuroelectrophysiology (NE) and clinical neuropathy in type 1 diabetes based on an exploratory analysis of the neuropathy data from the Diabetes Control and Complications Trial (DCCT). **Materials and Methods**: DCCT subjects who had a clinical neurological exam and NE testing at baseline and at year 5 (n=1238) were analyzed. Clinical neuropathy status was assessed by a neurologist and categorized as either definite, possible, or no neuropathy. We further classified the change in neuropathy at year 5 from baseline as being either worsened or non-worsened. Eight NE measurements (4 conduction velocity and 4 amplitude) were used in this analysis. Composite NE scores (scaled from 0-1) were developed for velocity, amplitude, and combining amplitude and velocity. **Results**: At year 5, 25.1% (n=311) showed a worsening of clinical neuropathy. Comparing the mean change in the nerve conduction velocity (NCV) measurements between those whose neuropathy worsened and did not worsen, it is found that those who worsened had a greater decrease in each nerve measured ($p<0.05$). Of the amplitude measurements, only the median sensory had a significant decrease associated with worsening neuropathy ($p=0.0141$). Modeling the eight NE measurements together, it is found that a one m/sec decrease in the peroneal motor velocity was significantly associated with a 4% increase in the worsening of clinical neuropathy (odds ratio=1.04, $p=0.0123$). In addition, the odds ratios associated with a 0.10 unit decrease in composite score were 1.10 for amplitude ($p=0.0191$), 1.08 for conduction velocity ($p=0.0329$), and 1.19 for the composite (velocity+amplitude) NE parameter ($p=0.0013$). **Conclusions**: These analyses showed that there was a significant relationship between the deterioration of nerve function and the worsening of clinical neuropathy. These exploratory results will encourage further investigation of the role of NE, particularly NCV in predicting deterioration of clinical neuropathy in patients with diabetes.

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SUBCLINICAL CEREBRAL LESION DEMONSTRATED BY MAGNETIC RESONANCE IMAGING IN INSULIN-DEPENDENT DIABETIC PATIENTS.

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Aims: To investigate the occurrence of parenchymal brain and spinal cord alterations in insulin-dependent diabetic patients by magnetic resonance imaging (RMI), we evaluated neuroimaging findings and studied the putative relationship between structural brain abnormalities and motor evoked potentials (MEP) and nerve conduction velocity (NCV) at ulnar, peroneal and sural nerves. **Materials and Methods:** MRI, MEP and VCN were performed on 20 asymptomatic patients (12 male, 8 female), aging from 21 to 48 years (mean ± SD: 34.3 ± 7 years). Diabetes duration was 21 ± 7 years. None of patients was receiving antihypertensive therapy or had evidence of macrovascular disease. Age, blood pressure, serum lipids, and HbA1c levels were correlated with results. **Results:** Nine patients (45%) had abnormal scans. Two of them showed central MEP dysfunctions. Six (30%) had peripheral neuropathy. Different types of abnormalities were observed: periventricular hyperintense white matter lesions (width > 2 mm) on T2w images in six patients; ventricular dilatation in eight patients; atrophic cortical changes in six patients; and lacunar infarcts < 2 mm and/or subcortical or basal ganglia ischaemic lesions in four patients who were older 40 years of age. Asymptomatic lacunae were correlated with age, but not with blood pressure, serum lipids, and metabolic control. Periventricular hyperintense white matter lesions in diabetes were not correlated with age, while they increase with age in nondiabetic population. **Conclusions:** These findings suggest that in insulin-dependent diabetes of long duration there are structural abnormalities in the brain which may represent accelerated neural aging associated with the metabolic disorder. These degenerative disturbances may result in future cerebrovascular damage.

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ATTENUATED IMMEDIATE EARLY GENE RESPONSES IN THE DIABETIC BB/W RAT PRECEDE IMPAIRED NERVE REGENERATION

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Nerve fiber regeneration is impaired in diabetic neuropathy and contributes to the progressive nerve fiber loss. The precise mechanisms responsible for this abnormality are not completely understood, although impaired neurotrophic support has been implicated. Previous studies from our laboratory have demonstrated attenuation of macrophage recruitment and delayed initiation of axonal regeneration in the diabetic BB/W-rat following sciatic nerve injury. **Aims:** Based on these data, we hypothesized that perturbed immediate early gene responses in Schwann cells may underlie these abnormalities, since they are believed to initiate macrophage recruitment and secondarily upregulate neurotrophic factors such as NGF and IGF-1. **Materials:** We examined the mRNA expression of the immediate early gene responses of IGF-1, NGF and their receptors IGF-1R and p75 at time points 0.5 hr to 4 days in 6-wk diabetic BB/W rats following sciatic nerve crush injury. The data were compared with those of non-diabetic control rats. **Results:** The immediate upregulation of NGF and IGF-1 peaked at 0.5 and 6 hrs after crush injury respectively in control nerves and was delayed to 24 hrs for both NGF and IGF-1 in diabetic nerves. The expression of p75 receptor was significantly attenuated in diabetic nerves. The baseline expression of IGF-1R in unsevered nerve was upregulated in diabetic nerves. However, in control nerve the expression of IGF-1R was immediately upregulated at 0.5 hr following nerve injury, while in diabetic nerves no upregulation was seen after crush injury. **Conclusion:** Attenuated immediate early gene responses of NGF and IGF-1 and their receptors may be responsible for the perturbed macrophage recruitment and initiation of axonal regeneration in diabetic nerve.

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EXPRESSION OF INSULIN AND IGF-I RECEPTORS' mRNAs IN PERIPHERAL NERVE OF TYPE 1 DIABETIC BB/W-RAT: EFFECTS OF C-PEPTIDE.

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Recent studies have demonstrated that C-peptide replacement in type 1 insulin-deficient diabetic BB/W-rats prevents nerve conduction velocity (NCV) deficits and inhibits hyperalgesia without altering glycemic levels and body weights.

Aims: Since C-peptide phosphorylates both the insulin receptor (IR) and the IGF-I receptor (IGF-IR), we examined the effects of C-peptide supplementation on the expression of the IR and IGF-IR in peripheral nerve.

Materials: Sciatic nerve from non-diabetic control (n=8), 2-mo diabetic (n=8), and 2-mo C-peptide treated diabetic BB/W-rats were examined immunohistochemically and by in situ hybridization with respect to IR and IGF-FR.

Results: Immunohistochemistry of sciatic nerve showed that the IR was localized to paranodal Schwann cells and to the nodes of Ranvier, whereas the IGF-IR was localized to the perinuclear cytoplasm of Schwann cells. Endoneurial microvessels were immunopositive for both receptors. In 2-mo diabetic insulin- and IGF-I-deficient BB/W-rats, in situ hybridization showed marked up-regulation of IR and IGF-IR expressions in Schwann cells and endothelial cells of endoneurial microvessels. These abnormalities were associated with decreased Na+/K+-ATPase activity and a 33% reduction in NCV ($p<0.0001$). Treatment with C-peptide in diabetic BB/W-rats resulted a normalization of the IR and IGF-IR expressions, accompanied by a 24% improvement in NCV ($p<0.0001$) and as previously demonstrated a 52% improvement in Na+/K+-ATPase activity ($p<0.005$).

Conclusions: These findings, in conjunction with our previous data demonstrating that C-peptide phosphorylates both the IR and IGF-IR, suggest that in the absence of insulin and decreased IGF-I in type 1 diabetic nerve, C-peptide can via its action on the IR and IGF-IR partially restore Na+/K+-ATPase activity and NCV.

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EFFECT OF THE INHIBITORS OF PKC ON SEROTONINERGIC TRANSMISSION IN STREPTOZOTOCIN - INDUCED DIABETES

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There is little data on the development of diabetic cerebral disorders. Aims: the study was undertaken to assess the inhibitors effects of PKC on serotoninergic transmission in diabetes. Materials and methods: we have studied the [2-^{14}C]serotonin uptake and release by brain cortex synaptosomes of 3 rat groups: control and another two after induction of diabetes by STZ (45 mg/kg, i.v.), one treated by an extract from a food plant containing natural inhibitors of PKC (5 mg/kg,, i.p., 7 days) the other untreated. Results: blood glucose levels were in control (6,8±0,6) and in diabetes (16,5±0,8mmol/l, p<0,05). The serotonin levels of brain cortex (ng/g tissue) were lower in diabetes: 0,234±0,012 vs 0,328±0,026 in control (p< 0,05). The finding sho-wed that [2-^{14}C]serotonin uptake was decreased by 38% in diabetes as compared to control. 1 μmol NAD (serotonin transmission modulator) treated synaptosomes demonstrated normalization of [2-^{14}C]serotonin uptake under diabetes It has been found that in vitro pretreatment of synaptosomes by H7 [1-(5-isoquinolinylsulfonyl)-2-methylpiperazine] (0,1 mmol/l, 20 min) elevated the serotonin uptake by 45% in diabetes. The study on the associated action of H7 and NAD failed to obtain any marked effect on [2-^{14}C]serotonin uptake as compared to that of H7. [2-^{14}C]serotonin release was found to be stimulated on 39% under diabetes as compared to control. Effect of NAD was activating both on diabetic and control rats synaptosomes. Exposure of H7 to synaptosomes caused further increase in [2-^{14}C]serotonin release on 20% under diabetes and 27% in control as compared to their groups. No significant effect of NAD additional to H7 action was observed. In rats treated with an extract from food plant [2-^{14}C]serotonin uptake was normalized, but neurotransmitter release remained elevated. Synergic effect of PKC inhibitor and NAD on serotonin release in vitro was determined. Conclusions: our results suggest that the beneficial effects of PKC downragulation on diabetic neuropathy are not due to normalization of brain serotonin content, but these effects may be related with changes in impaired transmission by the way peculiar to the action of NAD.

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Neuropathy – Treatment

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SYNERGY BETWEEN γ-LINOLENIC ACID AND ANTIOXIDANT EFFECTS ON DIABETIC RAT ERECTILE TISSUE DYSFUNCTION.

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Defective nitric oxide-mediated corpus cavernosum (CC) vasorelaxation is preventable by antioxidant treatment with α-lipoic acid (LPA) in diabetic rats, however, the dose required (300 mg kg^{-1} day^{-1}) is unrealistically high for patient use. Synergy between LPA and γ-linolenic acid (GLA) treatments was recently described for correction of sciatic neurovascular deficits. **Aims:** To determine whether combined low dose GLA-LPA treatment could protect CC neural and endothelial function in streptozotocin-diabetes. **Materials and Methods:** Diabetic rats were treated daily with equimolar doses of GLA (35 mg kg^{-1}) or LPA (28 mg kg^{-1}), alone and in combination, for 8 weeks. To study relaxation produced by nerve stimulation in vitro, CC were precontracted with phenylephrine, and preincubated with atropine and guanethidine to respectively abolish cholinergic and noradrenergic nerve responses. **Results:** Nerve electrical stimulation caused a relaxation that was blocked by nitric oxide synthase inhibition; characteristic of CC nitrergic innervation. Nondiabetic CC maximum neural relaxation was 80.6±4.6% (±SEM). This was reduced by diabetes (42.8±5.5%; p<0.001) and the deficit was unaffected by GLA or LPA treatments alone. Combined GLA-LPA treatment prevented the nitrergic relaxation deficit (77.7±3.9%; p<0.001). Endothelium-dependent relaxation to acetylcholine was completely blocked by nitric oxide synthase inhibition. Nondiabetic CC maximum acetylcholine relaxation (80.7±4.0%) was reduced by diabetes (52.2±2.8%; p<0.001). This was unaffected by LPA (51.6±4.4%) or GLA (53.5±6.0%) single-treatment. In contrast, the GLA-LPA combination completely prevented dysfunction (82.5±6.9%; p<0.001). **Conclusions:** A modest GLA-LPA dose prevented abnormal CC vasorelaxation in diabetic rats by a synergistic action: an approach that could be suitable for evaluation in clinical impotence trials.

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NERVE FUNCTION IN DIABETIC RATS: SYNERGY BETWEEN ALDOSE REDUCTASE AND ANGIOTENSIN CONVERTING ENZYME INHIBITION

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Reduced nerve perfusion and conduction velocity (NCV) in diabetic rats depends on mutually exacerbating vasa nervorum abnormalities including a deficit in nitric oxide-mediated vasodilation and elevated vasoconstrictor activity in the renin-angiotensin and endothelin systems. Vascular nitric oxide defects have been linked to aldose reductase inhibitor (ARI) correctable polyol pathway hyperactivity. **Aims:** To examine whether reducing vasoconstriction by angiotensin converting enzyme inhibitor (ACEI) treatment would interact additively or synergistically with an ARI to improve nerve function. **Materials and Methods:** After 6 weeks of streptozotocin-diabetes, rats were treated daily for 2 weeks with low doses of lisinopril (ZENECA Pharmaceuticals; 0.3 mg kg^{-1}) and ZD5522 (ZENECA; 0.25 mg kg^{-1}), alone or combined. **Results:** A 20.9±0.8% (±SEM; p<0.001) diabetic sciatic motor NCV deficit was 19.9±2.0% and 22.4±2.9% (p<0.001) corrected by ZD5522 and lisinopril, respectively. With combined treatment, correction was 96.1±3.1%, NCV markedly exceeding (p<0.0001) the predicted value from single treatment data (assuming additive drug effects). Sciatic endoneurial blood flow, 50.7±3.1% (p<0.001) reduced by diabetes, was not significantly altered by ZD5522 or lisinopril alone (15.0±5.1% and 10.0±9.0% correction, respectively). In contrast, perfusion was in the nondiabetic range for joint treatment; blood flow was much greater (p=0.0001) than predicted from the single treatment data. Lisinopril did not alter a 14.8-fold (p<0.001) diabetic elevation in nerve sorbitol levels. ZD5522 reduced sorbitol by 43.2±5.5% (p<0.001) and this was unaffected by joint lisinopril treatment (48.5±6.3%). **Conclusions:** A synergistic ARI /ACEI interaction was found that may be therapeutically advantageous in diabetic neuropathy and requires assessment in clinical trials.

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THE EFFECT OF GAMMA-LINOLENIC ACID-LIPOIC ACID ON FUNCTIONAL DEFICITS IN THE CENTRAL NERVOUS SYSTEM IN DIABETIC RATS

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Aims: Previous studies have shown that diabetic rats develop functional deficits in the central nervous system (CNS) after a diabetes duration of at least 2 to 3 months. The aim of the present study was to examine whether gamma-linolenic acid-lipoic acid (GLA-LA), a farmacon with beneficial effects on peripheral diabetic neuropathy, could reverse established functional deficits in the CNS in streptozotocin-diabetic rats. **Materials and Methods:** Treatment was initiated after 4 months of untreated diabetes and continued for 3 months. Effects on peripheral nerves were monitored by monthly measurements of motor and sensory nerve conduction velocities in the sciatic nerve (MNCV,SNCV). Effects on the CNS were monitored every 3 weeks by brainstem auditory evoked potential latencies (BAEP) and visual evoked potential latencies (VEP), which reflect impuls conduction velocity. At the end of the study, long term potentiation (LTP) was measured in hippocampal slices in vitro. Hippocampal LTP is widely used as a model for the neuronal mechanisms underlying learning and memory. **Results:** Significant deficits were observed in MNCV, SNCV, BAEP interpeak III-V latencies, VEP n3 latencies and LTP in diabetic rats compared to controls (ANOVAR $p < 0.001$). Treatment with GLA-LA after 4 months of untreated diabetes did not ameliorate the established deficits in MNCV and SNCV, nor in BAEP interpeak III-V latencies and VEP n3 latencies. However, GLA-LA did restore 65% of the LTP deficit in diabetic rats. **Conclusions:** GLA-LA had a beneficial effect on the hippocampal LTP deficit at a stage in which established deficits in parameters reflecting peripheral and central conduction velocity could not be reversed.

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IDD 676, A POTENT ALDOSE REDUCTASE SPECIFIC INHIBITOR PREVENTS NERVE DYSFUNCTION IN EXPERIMENTAL DIABETES.

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A wide variety of inhibitors of aldose reductase (hALR2) have been shown to prevent and even reverse peripheral nerve dysfunction in experimental diabetes. However, the majority of these inhibitors are non-specific, also inhibiting aldehyde reductase (hALR1). **Aims:** IDD 676 is a novel carboxylic acid which is selective for hALR2 (IC_{50} = 5 nM vs 27000 nM for hALR1). Consequently, the effects of IDD 676 on the motor nerve conduction velocity (MNCV) deficit of streptozotocin (STZ) diabetic rats were examined. **Materials and Methods:** In the first study, IDD 676 was administered by oral gavage for one month at doses of 5, 10 and 25 mg/kg/d. In the second study, the drug was administered in the diet at the same doses for three months. The primary efficacy variable, MNCV, was measured in the sciatic-tibialis nerve / interosseus muscle system. **Results:** The MNCV results indicate a clear dose-response improvement.

	Study 1 (m/s)	Study 2 (m/s)
Control	45.19 ± 1.90	48.13 ± 1.73
Diabetic	39.17 ± 1.61a	42.11 ± 2.11x
Diabetic + 25 mg/kg/d IDD 676	43.91 ± 3.18b	48.44 ± 3.94y
Diabetic + 10 mg/kg/d IDD 676	41.61 ± 2.49a	46.21 ± 2.33z
Diabetic + 5 mg/kg/d IDD 676	40.00 ± 1.54ac	45.10 ± 1.26

a : $p<0.01$ v control, b: $p<0.01$ v diabetic, c: $p<0.01$ v diabetic at 25mg/kg/d.
x: $p<0.001$ v control, y: $p<0.001$ v diabetic, z: $p<0.05$ v diabetic.
Data are mean ± SD, n = 6-10 per group and statistical analysis was one-way ANOVA with Tukey's range test.

Sciatic nerve sorbitol and fructose were completely normalized at all doses in both studies.

Conclusions: IDD 676, a potent selective inhibitor of aldose reductase, prevents the nerve dysfunction associated with diabetes at one month and three months in STZ-rats, a well established model for the prediction of human diabetic nerve dysfunction.

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ANTIOXIDANT TANACAN IN THERAPY OF DIABETIC NEUROPATHY

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Main aim of this study was to estimate effects of antioxidant treatment on diabetic neuropathy (DN).

Materials and methods. In 41 patients (mean age 57.52±9.19 yr., disease duration 10.58±8.06 yr.) the expressiveness of DN was assessed using Michigan Diabetic Neuropathy Score. Thiobarbituric acid reactive material was determined as malondialdehyde (MDA) in serum of all patients. Nerve conduction velocity (NCV) was determined in the median nerve in all patients. 25 patients received Tanacan (Egb 761, Beaufour-ipsen, France) in dosage 120 mg/day and 16 – placebo during 6 weeks.

	NIDDM	Placebo
MDA in serum, before and after	1.19±1.02	0.99±0.15
treatment (nmol/mg protein)	0.50±0.16 ***	0.95±0.22
MNDS, before and after treatment	9.56±2.61	7.65±2.48
(points)	2.92±2.31 ***	7.38±2.64
NCV, median nerve, sensor, before	48.25±5.54	58.53±7.58
after treatment (m/s)	55.37±3.62 ***	58.86±7.26
NCV, median nerve, motor, before	52.16±3.44	44.86±6.23
after treatment (m/s)	56.58±6.48 *	45.86±4.56
blood of velocity (mk/s), before	53.76±6.19	120.00±1.08
and after treatment	89.20±6.08 **	120.00±1.00

* P<0,05, ** P<0,01*** P<0,001 - before vs. after treatment

We conclude that Tanacan decrease oxidative stress, improve the blood supply and peripheral nerve functions in NIDDM patients with DN.

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AN ALDOSE REDUCTASE INHIBITOR AMELIORATES DIABETIC AUTONOMIC NEUROPATHY IN TYPE 2 DIABETIC SUBJECTS.

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Aims: Diabetic autonomic neuropathy (DAN) such as cardiovascular dysfunction may cause sudden death in diabetic subjects. Therefore, it is important to diagnose and to treat DAN at an early stage. In the present study, the usefulness of pupillary light reflex test, especially constriction rate (CR), as a diagnostic procedure of DAN and effect of an aldose reductase inhibitor, epalrestat, on DAN as well as diabetic somatic neuropathy (DSN) were investigated. **Materials and Methods:** Pupillary light reflex test was performed with 96 type 2 diabetic subjects and 51 non-diabetic subjects. Epalrestat was administered to 45 diabetic subjects with DSN for 6 months. Effect of epalrestat on CR was analyzed in relation to the severity of diabetic retinopathy. **Results:** 1) CR in diabetic subjects with DSN (0.21 ± 0.01, $p < 0.05$) was significantly lower than that in those without DSN (0.27 ± 0.03), which was also significantly reduced compared with that in non-diabetic subjects (0.34 ± 0.01). 2) Treatment with epalrestat significantly increased CR in diabetic subjects without retinopathy (0.27 ± 0.02 to 0.30 ± 0.01 $p < 0.05$) and those with simple diabetic retinopathy (0.27 ± 0.02 to 0.31 ± 0.02 $p < 0.05$), but not in those with preproliferative or proliferative retinopathy. 3) Epalrestat also significantly shortened the prolongation of the latency of F wave in median and tibial nerves. **Conclusions:** These observations suggest that CR in pupillary light reflex test may be an useful indicator of DAN, and that an aldose reductase inhibitor may have a therapeutic value for the early stage of DAN as well as DSN.

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BIMOCLOMOL ACCELERATES NERVE REGENERATION AFTER FREEZE LESION IN HEALTHY AND DIABETIC RATS

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The time course of the regenerative process after freeze lesion (F) to left sciatic n. and the curative effect of bimoclomol (20 mg kg^{-1} p.o. for 1-month) were studied in 4 groups of Crl. WI rats: treated (D+B) and untreated streptozotocin-diabetic (D), treated (C+B) and untreated controls (C). To assess nerve regeneration (NR) the return of nociceptive function of the footsole defined by hindlimb flexor reflex electromyogram (FR-EMG) was recorded weekly: before, at week (W) 3 of untreated diabetes, immediately and 2-6W from F, under anesthesia. Diabetes alone induced about a 30% (contralateral leg) suppression of FR-EMG area, due to sensory neuropathy, which was deepened by regenerative deficit to 98% after F (ipsilateral leg). Reappearance of EMG at W2 indicated that muscle reinnervation started. Nonlinear mathematical analysis revealed a slower recovery in D vs C at each week but the day 17 when the rate is maximal. The degree of regeneration reached 64% or 93% ($p<0.000$), respectively by W6. Improvements by 73% or 85% (for C or C+B, resp.) and 54% or 74% (for D or D+B, resp.) were achieved by W4. Bimoclomol accelerated NR in both the control (by about 1 week) and diabetic (approaching the control value) rats. Results may be explained by heat shock protein coinducing activity of bimoclomol in the cummulative disadvantageous case of diabetic and mechanically-induced sensory neuropathies.

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Autonomic Neuropathy – Monitoring and Analysis

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24-HOUR AMBULATORY BLOOD PRESSURE MONITORING AND ECHOCARDIOGRAPHIC EVALUATION IN TYPE 2 DIABETES.

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Aims: Comparing the 24-h BP profiles, and systolic and diastolic left ventricular function between micro- and normoalbuminuric type 2 diabetic patients in the absence of hypertension. Materials and Methods: 20 normoalbuminuric(NA-group) and 16 microalbuminuric(.MA-group>20µg/minute) normotensive patients with HbA1c levels <7.5% matched for age, sex, BMI, duration of diabetes, lipid profile, type of therapy were evaluated. 12-h daytime and nighttime urine samples were collected for measurements of microalbuminuria. Results: Mean day/night systolic BP values of NA-group were significantly higher than the MA-group (1.09±0.09 vs. 1.02±0.06, p=0.013).There wasn't a difference for diastolic dysfunction (relaxation abnormality-E/A<1) rate between the two groups (p=0.821). When patients were seperated into two groups according to their nighttime microalbuminuria, irrespective of total and daytime microalbuminuria, all of the 10 patients with nighttime microalbuminuria were non-dippers (<10%reduction in systolic BP) with respect to 61.5% in the remaining 26 patients (p=0.035). Patients with nighttime microalbuminuria had a higher incidence of retinopathy (30% vs. 3.8%, p=0.057). Patients with nighttime microalbuminuria had a higher night systolic BP (109.5±6.0mmHg vs. 101.8±12.1mmHg, p=0.066). When groups were reestablished according to dipping regardless of microalbuminuria, non-dippers had significantly higher 24-hour systolic (110.3±6.3mmHg vs. 103.5±8.8mmHg, p=0.013)and diastolic BP (79.0±5.1mmHg vs. 75.1±5.1mmHg, p=0.044) measurements than dippers. Non-dippers also had significantly higher nighttime microalbuminuria than dippers(16.8±16.3, median 12.8 vs. 4.7±5.4, median2.2, p=0.026).Conclusions: Nighttime urine samples are practical and can be more reliable than daytime urine samples. Diastolic dysfunction is not found to be related to microalbuminuria in normotensive type 2 diabetic patients.

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EVALUATION OF 24-HOUR HOLTER ECG COMPARED WITH AUTONOMIC FUNCTION TESTS IN DETECTING DIABETIC CARDIOVASCULAR AUTONOMIC NEUROPATHY

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Aims: To evaluate the diagnostic validity and reliability of 24-hour heart rate variability (HRV) compared with autonomic function tests (AFTs) in the detection of diabetic cardiovascular autonomic neuropathy (CAN) which carries an increased risk of mortality. **Materials and Methods:** We evaluated statistical (SDNN index, CV, sNN50, RMSSD), geometric (modified triangular index, triangular interpolation (TINN), top angle index [TAI]), frequency domain (spectral power in the VLF, LF, and HF bands, LF/HF ratio), and nonlinear measures (CV1 and CV2 of the Poincaré plot) of 24-hour heart rate variability (HRV) using Holter ECG as well as cardiovascular reflexes using AFTs in 95 healthy control subjects (C) and 125 diabetic patients. According to previously suggested definitions based on AFTs (>2 out of 7 indexes abnormal), 48 patients had definite CAN, 16 had borderline CAN, and 61 had no evidence of CAN. **Results:** The sensitivity of the Holter parameters was higher during the day (6:00-24:00 hours) compared with the night (00:00-6:00 hours) ($p<0.05$). The nonlinear measures of Poincaré plot were less sensitive in detecting CAN than the statistical (CV), geometric (TAI), and frequency domain (VLF, LF) indexes of 24-hour HRV (all $p<0.05$). Definite CAN defined on the basis of both these four indexes (>1 out of 4 abnormal) and AFTs was detected in 34% of the patients, while 50% had no evidence of CAN. However, while 13% of the patients had definite CAN detected by 24-hour HRV but no evidence of CAN using AFTs, the vice versa situation was found in only 3% of the patients, giving a difference of additional 10% of the total group who could be detected as having CAN only by 24-hour HRV. The coefficients of variation of day-to-day reproducibility in both healthy and diabetic subjects were lower for the statistical (CV) and geometric (TAI) parameters (2-9%) than for the frequency domain indexes (11-31%) and AFTs (22-76%). **Conclusion:** The combination of statistical, geometric, and frequency domain parameters of 24-hour HRV appears to be more sensitive and reliable in detecting CAN than autonomic function testing. The prognostic value of reduced 24-hour HRV in diabetes remains to be established.

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AUTONOMIC FUNCTION AND 24-H AMBULATORY BLOOD PRESSURE PROFILES IN PATIENTS WITH SHORT-LASTING TYPE 1 DIABETES

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Aim: to investigate whether autonomic function and diurnal blood pressure is associated with metabolic control in patients with short-lasting type 1 diabetes.

Materials and Methods: 41 diabetics were divided into two groups. Group 1 consisted of 18 pts with HbA1c ≤7% (av. 6.1±0.8%), while Group 2 consisted of 23 pts with HbA1c >7% (av. 9.3±2.0%). The two groups did not differ significantly as for age (23±5 vs 25±7 yrs), BMI (21.7±2.5 vs 22.8±3.4 kg/m^2), and duration of diabetes (3.2 ±0.9 vs 3.7±1.0 yrs). All subjects underwent 24-h ambulatory blood pressure monitoring (ABPM) and spectral analysis of heart rate variability.

Results: The analysis of group data showed significant differences in some of the autonomic nervous system indices and parameters obtained from diurnal blood pressure and heart rate profiles.

GROUP	HbA1c ≤ 7.0%	HbA1c > 7.0%	p
Mean RR interval - supine [ms]	954±120	862±127	0.025
Mean RR interval - upright [ms]	655±93	613±85	n.s.
Ln LF / Ln HF - supine	0.94±0.16	1.08±0.18	0.015
Ln LF / Ln HF - upright	1.31±0.21	1.47±0.33	n.s.
SBP-24h [mmHg]	117±10	121±9	n.s.
DBP-24h [mmHg]	69±6	71±6	0.022
HR-24h [beats/min]	74±10	82±9	0.009
SBP-day [mmHg]	121±11	127±9	n.s.
DBP- day [mmHg]	74±6	78±7	0.034
HR- day [beats/min]	79±12	88±11	0.019
SBP-night [mmHg]	106±9	108±9	n.s.
DBP-night [mmHg]	58±7	62±6	n.s.
HR- night [beats/min]	63±10	68±10	n.s.

Conclusions: Our data indicate that glycaemic control significantly influences autonomic nervous system function and blood pressure levels already in short-lasting type 1 diabetes.

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SPECTRAL POWER ANALYSIS IN REAL TIME: AGING AND DIABETES RELATIONSHIP.

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With a view to studying heart rate variability we used a spectral power analysis, obtained through acquisition and elaboration of a common ECG-signal in real time. Instrumentation and analysis procedures (hybrid algorithm achieved by means of linear data prediction, followed by Fourier analysis) were modified in order to obtain significant parameters: Total Power (PT) and LF/HF ratio, starting from temporal fragments, characterized by a stationarity condition.
We studied 91 subjects, divided into 4 groups: 28 IDDM (group A), 19 NIDDM > 65 yrs old (group B), 23 young controls (group C), and 21 old controls , > 65 yrs old (group D). NIDDM group was free from other diseases.
The PT value is expressed in arbitrary units.
Results:

	N.	Mean Age	Mean PT	Mean LF/HF
Group A	28	28 +/- 2	21000 +/-23000	4,4 +/- 5,1
Group B	19	69 +/- 7	9000 +/- 6000	2,8 +/- 1,9
Group C	23	28+/- 8	36000 +/- 21000	4,7 +/- 4,8
Group D	21	73 +/- 6	15000 +/- 7000	3,6+/- 5,3

Data analysed by ANOVA are statistically significant (p<0,01) and show:

- Normal PT threshold is PT > 10000.
- Young subjects present an increased PT and PT variability with respect
- to older ones.
- The increased variability of IDDM correlates with Ewing Test Score (ETS).
- Reduced NIDDM variability is not strictly correlated to ETS.
- PT and ETS seems to be age -related.

Total Power of Real Time Spectral Analysis may be proposed as a new measure of Autonomic Nervous System integrity.

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REPRODUCIBILITY OF STAGING OF AUTONOMIC DYSFUNCTION IN DIABETES USING HEART RATE VARIABILITY ASSESSMENT

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Short-term spectral analysis of heart rate variability (SA-HRV) bears potentially more information on autonomic control of the heart than standard bedside tests (Ewing's battery). However, its reproducibility -- particularly in different stages of autonomic dysfunction -- has not been sufficiently delineated yet. **Aims:** Evaluation of short-term reproducibility of SA-HRV and Ewing's battery in staging of cardio-vascular autonomic neuropathy (CAN) in diabetes. **Patients and methods:** Intrain-dividual comparison of 2 consecutive measurements of HRV in frequency- (modified orthostatic load: supine-standing-supine, 3x5 minutes) and time-domain (Ewing's battery), recorded in 55 diabetic patients with various stages of CAN (*no/early/severe* CAN: n=21/22/12), within 1.2±0.9 days, using a telemetric system *VariaCardio TF4*. Examination conditions were strictly standardised. Correlation coefficients (CC) and coefficients of reproducibility (CR) were calculated. **Results:** In frequency-domain analysis of whole population, paired correlation test delivered the highest CC for LN *cumulative* spectral power in HF band (r=0.95, p<0.001), in total frequency band (r=0.94, p<0.001) and in LF band (r=0.90, p<0.001), whereas in VLF band correlation was less manifested (r=0.74, p<0.001). When considering the results separately at different stages of autonomic dysfunction, in groups with *no* and *early CAN* the highest intraindividual CC was reached for cumulative spectral power in HF band (r=0.91; r=0.84; both p<0.001), in a group with *severe* CAN the highest CC was recorded in LF band (r=0.75, p<0.001). Similarly, the best CR were recorded for LN *cumulative* spectral power of LF+HF bands (CR=0.9 ms^2), HF (CR=1.1 ms^2) and LF (CR=1.2 ms^2) bands. In time-domain analysis of HRV, the highest CC was found for deep breathing test (I-E: r=0.88, p<0.001; CR=9.6 min^{-1}), Valsalva manoeuvre (VR: r=0.82, p<0.001; CR=0.29) and orthostatic load (HRmax/min r=0.80, p<0.001; CR=0.34), whereas blood pressure difference induced by orthostatic load delivered lower correlation (r=0.58, p<0.01; CR=49 mm Hg). **Conclusions:** In our study, results of short-term measurements of HRV in time and frequency-domain if repeated under standardised conditions within 2 days remain stable and deliver high correlation coefficients and sufficient coefficients of reproducibility.

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PREVALENCE OF QTc PROLONGATION IN TYPE 2 DIABETIC: AN ITALIAN POPULATION-BASED COHORT

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A prolonged QT interval is associated with sudden death and poor survival in healthy subjects and in type 1 diabetic patients: the prevalence of QT prolongation in type 2 diabetic patients is not known, even if they represent the majority of diabetic patients in the community. **Aims** of the present study were to evaluate the prevalence of QT interval prolongation in a population-based cohort of type 2 diabetic patients and its relationship with sex, age, diabetes duration, glycemic control, blood pressure and IHD. **Patients and methods:** the study base for this report were all 1354 patients with known Type 2 diabetes on the prevalence date (1 October, 1988) resident in Casale Monferrato, a rural area in the north-west of Italy, with 93,477 inhabitants. Patients were studied to obtain the following: height and weight, blood pressure fasting plasma glucose, triglycerides, total cholesterol, HDL-cholesterol, fibrinogen, HbA1c, albumin excretion rate (AER), smoking habit. A standard supine 12-lead ECG was recorded and coded according to the Minnesota code criteria in order to define probable or possible IHD. RR and QT intervals were measured in five consecutive cycles and the QT interval corrected for the previous cardiac cycle length (QTc) was calculated according to the Bazzet's formula QTc=QT/RR ^{½}. QTc > 0.44 s was considered abnormally prolonged. **Results:** the prevalence of abnormally prolonged QTc was 26.1% (95% CI 23.8-28.4), with no sex differences (men: 23.5%, 95% CI 20.1-26.9; women: 28.0%, 95% CI 24.9-31.1). In both sexes, patients with IHD had higher mean values of QTc and higher adjusted prevalence of prolonged QTc. In contrast, no differences were found either in prevalence of QTc > 0.44 sec nor in mean values of QTc, by AER and antidiabetic treatment. In hypertensive women only, the prevalence of QTc > 0.44 sec was significantly higher than in normotensive women (17.6% vs. 29.4%, p=0.03). In **conclusion**, the present population-based study has shown that the prevalence of QTc prolongation in type 2 diabetic patients is considerable high (26%), and that it is associated with IHD.

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ANALYSIS OF QTc INTERVAL IN TYPE-2 DIABETIC PATIENTS WITH OVERT NEPHROPATHY

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Aim: To evaluate the prevalence of long QTc interval in type-2 diabetic patients with and without established diabetic nephropathy. **Patients and Methods:** 46 patients with overt nephropathy (N) (proteinuria > 0,5g/24h; age 66,7±8,2 yr; diabetes duration: 21,0±6,9 yr) and a minimum diabetes duration of 10 years were compared with 40 patients without nephropathy (C) (proteinuria <0,5g/24h; age 65,9±9,8 yr; diabetes duration: 23,1±7,1 yr). Along with the assessment of usual clinical and biochemical variables, QTc was calculated using Bazet's formula on lead D-II of a standard 12-lead electrocardiogram (25 mm/sec) as the average of three consecutive beats. **Results:** Mean QTc did not differ between groups (N: 436,7±39; C: 434,3±41,8; T-test NS). Prevalence of long QTc (>440) was not different between groups (N: 41,3%; C: 42,5%; χ^2-test NS). Bivariate analysis using all patients as a whole group showed that the only parameters associated with long QTc were the presence of macroangiopathy (72,2% χ^2-test p <0,005) and treatment with calcium channel blockers (57,1% χ^2-test p<0,02); on the other hand a slight correlation was observed between QTc and K^+ levels (r: -0,293; p <0,01). In the logistic regression analysis only the presence of macroangiopathy remained significant (p<0,02). **Conclusion:** The prevalence of prolonged QTc interval is not a specific trait of type-2 diabetic patients with overt nephropathy. As an additional risk factor, prolonged QTc interval is associated with macroangiopathy in type 2 diabetic patients.

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QT-Interval Prolongation in Diabetic Subjects: Effects on Mortality

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It has been suggested that diabetic autonomic neuropathy resulting in QT-interval prolongation is associated with increased mortality. This hypothesis was tested prospectively in a cohort of 300 diabetics (age: 45.1±5.9 years m±SD; gender: 160 f, 140 m; duration of diabetes 11.4±8.3 years).

QT-intervals were measured on baseline EKGs by an experienced cardiologist using a digitizer (Calcomp). QT was corrected for the respective heart rate using Bazett's formula (QTc). Life/death status was assessed after 8, 13 and 23 years (±0.2). Survival analysis was performed using the Cox proportional hazards model with survival time as the dependent variable in the model.

After adjustment for age and duration of diabetes, QTc was a significant predictor of all-cause mortality after 13 and not after 23 years:

	Exp(Coef)	95%-CI	p
All subjects (13 years)	1.002	1.000-1.004	0.0226
All subjects (23 years)	1.001	0.999-1.003	0.2480

When analyzing patients with and without proteinuria at baseline separately, duration of QTc was a significant predictor of all-cause mortality only in patients with coexistent macroproteinuria (only after 13 years):

	Exp(Coef)	95%-CI	p
With proteinuria (13 years)	1.013	1.005-1.022	0.0022
No proteinuria (13 years)	1.001	0.999-1.004	0.3515
With proteinuria (23 years)	1.004	0.997-1.012	0.2705

Conclusion: These data obtained in a large cohort of diabetics show that prolongation of QTc is associated with increased all-cause mortality during the first 13 years. However, this effect is only seen in patients with coexistent macroproteinuria and does not persist in a long-term follow-up (23 years).

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Autonomic Neuropathy – Baroreflex and Orthostasis

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BAROREFLEX SENSITIVITY IS DEPRESSED IN TYPE-I DIABETIC PATIENTS COMPLICATED BY MICROALBUMINURIA

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Aims: To evaluate baroreflex sensitivity (BRS) in micro- and normoalbuminuric type 1 diabetic patients and healthy subjects without autonomic neuropathy.

Materials and methods: 15 Microalbuminuric type 1 diabetic patients (11 male, 4 female, age 50±11 yr (mean±SD), body mass index (BMI) 26.3±3.1 kg/m^2) were matched for age, gender, BMI and smoking habits with 15 normoalbuminuric type 1 diabetic patients and 15 healthy controls. All had a blood pressure <160/95 mmHg and no signs of neuropathy (standard autonomic function tests). Blood pressure and heart rate were measured non-invasively (Finapres) during rest and sympathetic activation (handgrip, mental stress, cold pressor, standing). The BRS was defined as the mean gain between blood pressure variability and heart rate variability in the 0.07-0.15 Hz frequency band. Differences between groups between were analyzed with ANOVA after natural log (LN) transformation.

Results: Resting BRS was decreased in microalbuminuric patients compared with normoalbuminuric patients and controls (p<0.001). All sympathetic tests reduced BRS in each group. However, the BRS differences between the groups persisted.

BRS	Rest	mean ± SEM	Standing	mean ± SEM
millisecs/mmHg	BRS	LN(BRS)	BRS	LN(BRS)
Controls	9.49	2.25 ± 0.10	5.99	1.79 ± 0.09#
Normoalb. type 1 DM	7.69	2.04 ± 0.19	4.48	1.50 ± 0.23#
Microalb. type 1DM	3.53	1.26 ± 0.11*	2.20	0.79 ± 0.20*#

All p-values were adjusted with Bonferroni correction. * p<0.001 Microalb. vs. controls and microalb. vs. normoalb. #p<0.01 Rest vs. standing, paired t-test.

Conclusions: In type 1 diabetic patients with microalbuminuria and no overt neuropathy, baroreflex sensitivity is evidently depressed during rest and sympathetic activation. This abnormality may play a role in the association between increase in urinary protein loss and cardiovascular disease.

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IS POOR DIABETIC CONTROL RELATED WITH MODIFIED CAROTID BAROREFLEX FUNCTION ?

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The aim of study was to assess if the duration and poor control of hyperglycaemia are associated with modified baroreflex mediated heart rate (HR) and mean arterial pressure (MAP) reactions in non-insulin dependent diabetes mellitus (NIDDM). Materials and Methods: On 18 pts with NIDDM (men, 61 ± 1.8 yr aged, Hb A_{1C} 10.1 ± 0.9%, ranged from 8.1 to 11.2% and duration of syndrome ranged from 4 to 16 yr) and 19 controls (gender and age matched) beat-to-beat HR and finger MAP were monitored non-invasively. Bradycardic and hypotensive reactions to baroreflex activation by neck suction (- 60 mmHg, for 5 s) were evaluated at rest, during handgrip for 60 s, with force 50% of maximal and during postexercise arterial occlusion for 1 min. All medications with the exception of hypoglycaemic agents were discontinuated 2 weeks before the study. Values are expressed as means ± standard error. Results: At rest HR (80 ± 2 vs 70 ± 3 bpm; P< 0.05) and finger MAP (113 ± 2.7 vs 97 ± 1.4 mmHg; P< 0.05) were increased and bradycardic (1.8 ± 0.3 vs 10 ± 0.6 bpm, P< 0.02) and hypotensive (3.5±0.7 vs 9.1±0.8 mmHg,P<0.05) reactions to baroreflex activation were reduced in NIDDM patients comparing to controls. At handgrip cessation moment the amplitude of pressor reaction was similar (29 ± 5 vs 26 ± 2 mmHg), but HR acceleration amplitude was decreased in NIDDM pts. comparing to controls (12.2 ± 2 vs 22 ± 2 bpm; p< 0.05), as well as smaller part of pressor reaction amplitude was supported by postexercise arterial occlusion (36 ± 8% vs 57 ± 3%; P< 0.05). The level of HbA_{1C} was inversely related to baroreceptor reflex mediated bradycardic reaction (r = - 0.68; P< 0.05) and hypotensive reaction (r=- 0.59; P<0.05) as well as to postexercise arterial occlusion pressor reaction amplitude (r = - 0.72; P< 0.05), but there were no found relationships between duration of diabetes and analysed baroreceptor reflex and pressor reaction parameters. Conclusions: In subjects with NIDDM cardiovascular autonomic dysfunction is rather related with the degree of hypoglycaemia than duration of diabetes.

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INFLUENCE OF PANCREATIC ISLET TRANSPLANTATION ON BAROREFLEX SENSITIVITY IN DIABETIC PATIENTS

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Aims: Pancreatic islet transplantation may improve the carbohydrate metabolism and delay the development of diabetic complications. The aim of this study was to analyze the *spontaneous* baroreflex sensitivity (BRS) in the resting supine position and after standing up in patients (pts) with long-standing insulin-dependent diabetes mellitus (IDDM) after islet cell transplantation (Tx).
Materials and methods: 10 Tx pts (age: 43.7±2.6 years, duration of IDDM: 26.4±2.7 years, graft function: 9.6±1.0 years; mean±SEM), 17 non-transplanted (n-Tx) IDDM pts (age: 46.9±3.8 years, duration of IDDM: 23.5±3.1 years) and 15 healthy controls (age: 46.0 ±1.7 years) were studied. The blood pressure was measured continuously with a *Finapres 2300* instrument (Ohmeda). The ECG signal was detected with a *Sirecust 730* (Siemens) electrocardiograph. The signals were fed through an analog-digital converter into a computer and analyzed off-line. The BRS was calculated in the supine position and after standing up.
Results: Both in the n-Tx and in the Tx group, the BRS was decreased in the supine position and also after standing up relative to the control value. In the Tx pts, the BRS values were elevated as compared with those for the n-Tx group. In the control group, the BRS was decreased after standing up in comparison with the resting position. In both the n-Tx and Tx groups, there was no significant difference between the BRS values measured in the supine and standing positions.

	Control	n-Tx	Tx
BRS in supine position (ms/mmHg)	8.7±0.9	*3.2±0.6	†*5.5±0.7
BRS after standing up (ms/mmHg)	‡5.3±0.5	*2.0±0.4	†*3.6±0.8

$p<0.05$ vs control, †$p<0.05$ Tx vs n-Tx, ‡$p<0.05$ standing vs supine position

Conclusions: 1) In patients with long-standing IDDM, a severe impairement of the cardiovascular adaptation was found. 2) The BRS was less diminished in the transplanted patients relative to the non-transplanted group. 3) Pancreatic islet transplantation may delay the progression of diabetic autonomic neuropathy.

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CARDIOVASCULAR MONITORING DURING TILT AND SQUATTING TESTS IN DIABETIC PATIENTS WITH AUTONOMIC NEUROPATHY

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Aims : To better understand the underlying mechanisms of orthostatic hypotension in diabetic patients with cardiac autonomic neuropathy (CAN). **Materials and methods** : The haemodynamic changes during a passive (tilt test 70°: Tilt) and an active (squatting to standing : Sq-St) orthostatic maneuver were compared in 9 diabetic subjects (D) with markedly decreased E/I R-R ratio during deep breathing (1.06 ± 0.02) and in 9 healthy controls (C). Simultaneous monitoring of systolic and diastolic arterial blood pressure by finger photoplethysmography (Finapres®) and of heart rate and stroke volume by bioimpedance cardiography (Kardiocom®) allowed to calculate mean arterial pressure (MAP), cardiac output (CO), systemic vascular resistances (SVR) and baroreflex gain. **Results** : When compared to C, D showed a greater fall in MAP (Tilt : - 9 ± 4 *vs* + 2 ± 2 mm Hg, p = 0.019; Sq-St : - 45 ± 8 *vs* - 17 ± 6 mm Hg, p = 0.009) and a dampened reflex tachycardia (Tilt : + 8 ± 1 *vs* + 15 ± 2/min; p = 0.017; Sq-St : + 13 ± 3 *vs* + 25 ± 3/min, p = 0.01) during the two orthostatic maneuvers. During Sq-St, the baroreflex gain was markedly lower in D than in C (1.22 ± 0.25 *vs* 4.49 ± 1.24 msec/mm Hg, p = 0.025). Whereas SVR remained almost stable whatever the posture in C, SVR decreased progressively during Tilt and more abruptly during Sq-St in D, suggesting a lack of appropriate vasoconstriction. Such a defect combined with insufficient CO adaptation explained the greater MAP fall during orthostatism in D. Differences were more evident during the squatting test which clearly appears to be more discriminant than the tilt test to assess autonomic dysfunction. **Conclusions** : Orthostatic hypotension of diabetic patients with severe CAN results from a passive adjustment of vascular resistances to posture changes, without compensatory sympathetic vasoconstriction and reflex tachycardia.

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BLOOD PRESSURE RESPONSE TO STANDING IN THE DIAGNOSIS OF AUTONOMIC NEUROPATHY: THE EURODIAB IDDM COMPLICATIONS STUDY

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Aims: Blood pressure (BP) response to standing is the most simple test to study sympathetic integrity. We intended to evaluate which diagnostic criteria of abnormal response should be considered as optimal. **Materials and Methods:** The EURODIAB IDDM Complications Study involved the examination of randomly selected type 1 patients from 31 centres in 16 European counties. Data from 3007 patients were available for the present evaluation. Responses of heart rate /30/15 ratio/ and BP from lying to standing just as the frequency of feeling faint on standing up were assessed. **Results:** 30/15 ratio was abnormal in 24% of patients. According to different diagnostic criteria of abnormal BP response to standing (› 30 mmHg, › 20 mmHg, and › 10 mmHg fall in systolic BP), the frequency of abnormal results were 5,9 %, 18 % and 32 %, respectively (p<0,001). The frequency of feeling faint on standing was 18%, thus, it was identical with the prevalence of abnormal BP response to standing when › 20 mmHg fall in systolic BP was considered as abnormal. Feeling faint on standing correlated significantly with both autonomic test results (p<0,001). **Conclusions:** A fall › 20 mmHg in systolic blood pressure after standing up seems to be the most reliable criterion for the assessment of orthostatic hypotension in the diagnosis of autonomic neuropathy.

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AUTONOMIC FUNCTION DURING A SQUATTING TEST IN TYPE 1 DIABETIC PATIENTS WITH OR WITHOUT MICROALBUMINURIA

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Aims : To better understand the relationships between diabetic nephropathy and autonomic cardiovascular neuropathy in type 1 diabetes. **Materials and methods** : Changes in mean arterial blood pressure (MAP) and heart rate were measured with a Finapres® (Ohmeda, USA) during an active orthostatic test (squatting test : SqT) in patients with long-standing (> 10 years) type 1 diabetes in function of incipiens nephropathy defined by repeated microalbuminuria (μA : ≥ 20-200 μg/l). **Results** : Sex ratio, age, diabetes duration (mean ± SD : 25.1 ± 9.5 *vs* 24.8 ± 8.7 yrs) and body mass index were similar in 30 patients without μA (7.0 ± 3.4 μg/l) and in 25 patients with μA (78.6 ± 49.5 μg/l). Mean HbA_{1c} levels during the last 4-8 years tended to be higher in patients with μA than in those without μA (8.4 ± 1.4 *vs* 7.8 ± 1.3 %, p = 0.07) and individual μA levels were positively correlated with HbA_{1c} concentrations (r = 0.32; p = 0.019). E/I R-R ratio during deep breathing (1.26 ± 0.14 *vs* 1.20 ± 0.15, NS) was similar in both groups. R-R SqT ratios defining "SqT vagal" *during* squatting was lower in patients without than in those with μA (0.83 ± 0.11 *vs* 0.90 ± 0.09, p = 0.01), in contrast to "SqT sympathetic" *after* squatting which was similar (1.17 ± 0.14 *vs* 1.13 ± 0.11, NS). Posture change from squatting to standing also induced similar transient orthostatic hypotension in patients without and with μA (- 20 ± 9 *vs* - 16 ± 9 mm Hg, NS). However, the baroreflex gain (slope of the regression line relating R-R intervals to MAP changes) was significantly lower in patients with μA (2.20 ± 1.88 msec/mm Hg) than in those without μA (3.36 ± 2.38 msec/mm Hg, p = 0.048). **Conclusions** : Only minimal cardiovascular dysregulation was detected during squatting in patients with microalbuminuria, suggesting that diabetic autonomic neuropathy and incipiens nephropathy partially result from different pathophysiological mechanisms.

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INCREASE IN THE NEUROENDOCRINE RESPONSE TO STANDING WITHIN IN THE FIRST THREE MONTHS OF TYPE 1 DIABETES IN MAN

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The aim of the study was to describe peripheral somatic and autonomic nerve function in diabetic patients during the first year after the diagnosis of type 1 diabetes. **Methods :** All patients with newly diagnosed type 1 diabetes submitted to our department during two years were invited to participate in the study. The patients were evaluated within 1 month and 3, 6 and 12 months after the diagnosis. At each visit the evaluation comprised history; clinical examination; blood tests; electrophysiology, biothesiometry and tests of heart rate variability. During the orthostatic test procedure pulse, blood pressure and p-catecholamines were measured at -2,0,1,3, and 5 minutes. **Results**: Six patients completed the study. Median Hb_{A1C} was 12%(10.0;12.8)(25% and 75% fractiles) at baseline. At three months median HB_{A1C} was 8,3 (7.8;9.0). After one year median HB_{A1C} had decreased to 7,9 (7.3;8.8). The maximal p-noradrenaline level (p-NA) during orthostatic test increased in all patients at three months after diagnosis and was significantly different from baseline ($p<0.05$, Wilcoxon matched pairs test (WMP)). At three months median maximal p-NA level was 0.21 ng/ml (0.16;0.28) versus 0.14 ng/ml (0.09;0.18) at baseline. Levels decreased 6 monthes after diagnosis ($p<0.05$ (WMP)) and remained stable 12 months after diagnosis. There was no significant difference between baseline and 12 month's maximal p-NA response. There was a tendency towards a increase in pulse raise during the orthostatic procedure at three months ($p=0.09$ (WMP)) but no changes in blood pressure response. No significant changes in somatic peripheral nerve function or heart rate variability could be detected. **Conclusion :** A significant increase in maximal p-NA during orthostatic test three months after diagnosis of type 1 diabetes was accompanied by a tendency towards increase in heart rate response during same procedure. This may reflect a compensatory increase in sympathetic nervous activity in response to a decrease in the intravascular volume following institution of insulin treatment.

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AUTONOMIC NEUROPATHY AND HYPERTENSION IN IMPAIRED GLUCOSE TOLERANCE: THERE IS A RELATIONSHIP

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Aims: Our previous studies provided evedence of relationship between autonomic neuropathy and hypertension both in patients with IDDM and NIDDM. In the present study we evaluated the possible connection between autonomic neuropathy and hypertension in patients with impaired glucose tolerance (IGT). **Materials and methods:** We examined 24 patients with IGT (mean age: 55,7 ± 10,8 ys; range: 40-79; males: 8; females: 16). The five standard reflex-tests of cardiovascular autonomic function were applied; twenty-four-hour-long blood pressure monitoring was performed by MEDITECH ABPM 02 device. Severity of CAN was characterized by the number of abnormal reflex indices on patient. **Results:** CAN was present in 16 from 24 patients, while hypertension was found in 9/24 patients by the 24-hour-long-blood pressure monitoring. All patients with hypertension had autonomic neuropathy. Severity of CAN correlated significantly with mean systolic ($p < 0,001$) and diastolic ($p < 0,01$) blood pressure values, just as with systolic ($p < 0,01$) and diastolic ($p < 0,05$) hypertensive time indices, and systolic and diastolic hyperbaric impact values (both $p < 0,05$). Severity of sympathetic autonomic neuropathy was significantly related to mean systolic and diastolic blood pressure values as well as to systolic and diastolic hypertensive time indices (both $p < 0,01$) and systolic and diastolic hyperbaric impact values (both $p < 0,05$). **Conclusions:** Autonomic impairment may be present in subjects with IGT and – as a novel finding - seems to be associated with hypertension in these patients. ABPM should be performed for the early assesment of hypertension in patients with IGT and autonomic neuropathy. Hypertension itself may contribute to the poor prognosis of CAN.

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NOCTURNAL BLOOD PRESSURE FALL AND HEART RATE VARIABILITY IN TYPE 1 DIABETES.

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Aims : To investigate the association between cardiac autonomic neuropathy assessed using spectral analysis of heart rate variability and nocturnal blood pressure (BP) fall in type 1 diabetes mellitus. **Patients and methods :** 59 type 1 diabetes mellitus patients (mean age : 42.1±11.9 years ; diabetes duration : 13.2±9.6 years) were studied. They were normotensive (BP<140/90 mmHg) and received no treatment other than insulin. Blood pressure variations were assessed using 24-h ambulatory blood pressure monitoring. The 59 patients were subclassified in 2 groups on the basis of nocturnal BP fall: Group 1: dippers with > 10% nocturnal reduction of SBP (N = 33) and Group 2 : nondippers with ≤ 10% reduction (N = 26). Power spectral analysis of RR interval variability was applied to ECG recordings to obtain for 24 hours, day and nighttime periods total power (TP : 0-2 Hz), low frequency power (LFP : 0.039-0.148 Hz) and high frequency power (HFP : 0.148-0.348 Hz). **Results :** Patients of group 2 were older than those of group 1 (45.7±12.5 vs 37.9±10.6 years, $p<0.01$). Body mass index, duration of diabetes, HbA1c and tobacco consumption did not differ between the 2 groups. Nondippers clinical SBP and DBP were higher than those of dippers but differences were not significant. The mean 24h BP and the nighttime BP were higher in nondippers than in dippers (nighttime SBP: 115±10 vs 101±10 mmHg, $p<0.001$; nighttime DBP: 67±6 vs 60±6 mmHg, $p<0.001$). There was no correlation between SBP nocturnal fall and patients' age or diabetes characteristics. Nondippers had lower HFP and LFP than dippers but the difference was significant on the 3 periods only for LFP. **Conclusions :** In type 1 diabetes, diminished nocturnal BP fall is associated with a decrease in LF. This association could explain the poorer prognosis for cadiovascular events.

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MELATONIN RHYTHMS IN TYPE 2 DIABETES MELLITUS WITH RESPECT TO CARDIAC AUTONOMIC FUNCTIONS:

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AIMS: The present study has been designed to determine the correlation of the diurnal rhythm of melatonin secretion with respect to the cardiovascular autonomic functions in type 2 diabetes mellitus. **MATERIALS AND METHODS:** A total of 36 subjects with type 2 diabetes mellitus and 17 healthy subjects were recruited for the study. Diurnal rhythm of melatonin secretion was assessed by measuring serum melatonin concentrations between 02:00- 04:00 a.m. during sleeping and 16:00-18:00 p.m. Evaluation of the cardiac autonomic nervous system functions of the diabetic group included the analysis of heart rate variability by frequency-domain analysis and 24-hour blood pressure monitoring. Separate analysis of the diurnal variation of the low and high frequency powers and the ratio of these components which determine the sympathovagal balance was performed and compared with the melatonin secretion patterns. Pineal gland imaging was carried out by magnetic resonance imaging and computed tomography.**RESULTS:** Diurnal rhythm of melatonin secretion was blunted in the diabetic group compared to the nondiabetic group ($p= 0.03$). Comparison of the diabetic and the nondiabetic group showed significant difference in night to day low frequency power ratio ($p= 0.037$). Diurnal rhythm of melatonin secretion of the diabetic group was found to be correlated significantly to the night to day low power frequency ratio ($p=0.04$) in univariate analysis. 95 % of the diabetic patients showed calcification of the pineal glands whereas frequency of calcification reaches 70 % in healthy controls.**CONCLUSIONS:** This study shows that diurnal melatonin secretion pattern of type 2 diabetic patients is blunted when compared to the healthy controls and melatonin secretion seems to be correlated to the night to day low power frequency ratios analyzed by frequency domain analysis of heart rate variability. It was also documented that pineal gland calcification is more frequently encountered in the diabetic patients.

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DISTURBANCES IN AUTONOMIC BALANCE AND DIABETIC LATE COMPLICATIONS

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The aim was to assess the autonomic balance in healthy and 1-type diabetic patients with and without diabetics late complications (retinopathy, neuropathy, nephropathy). To evaluate the pattern of cardiovascular autonomic balance standard cardiovascular reflex tests (Valsalva, Ewing, deep-breathing) and short rate variability were performed. Using computer assisted method (ProSciCard, Linden, Germany) tests were performed in 23 healthy persons (control group -C) and in 34 diabetics without (D0) and in 55 diabetics with late complications (D1). The power spectrum of heart rate variability (HRV) was determined in very low (VLF: 0.01-0.05 Hz), low (LF: 0.05-0.15 Hz) and high (HF: 0.15-0.50 Hz) frequency ranges at rest (R), after active standing (S), after deep breathing (B), after cold pressure test (CPT). Absolute and relative (%) spectral analysis HRV power were assessed by Fast Fourier Transform. The autonomic balance were estimated by ratios: VLF/LF, LF/HF.

Results:

The following parameters had the highest strenght of discrimination between examined groups:

C vs D0: In LF-S/HF-S (-0.46 vs 0.35; $p<0.01$), In VLF-B/LF-B (-0.42 vs -0.0003; $p<0.001$), In VLF-CPT/LF-CPT (-0.52 vs 0.007; $p<0.001$) and In LF-CPT/HF-CPT (-0.31 vs 0.22; $p<0.01$).

D0 vs D1: In VLF-R/LF-R (0.05 vs 0.55; $p<0.01$); In LF-S/HF-S (1.59 vs 1.07; $p<0.05$), In VLF-D/LF-D (-0.0003 vs 0.69; $p<0.001$), In VLF-CPT/LF-CPT (-0.007 vs 0.41; $p<0.01$).

Disturbances in autonomic balance develop in parallel to diabetic late complications and might account for increased mortality risk in type I-diabetics. Measurement of autonomic dysbalance after simple physiological tests might be a method worth of application in future diagnosis of diabetic patients.

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DIABETES AFFECTS THE RELATION BETWEEN AUTONOMIC NERVOUS FUNCTION AND SURVIVAL IN THE ELDERLY

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Aim: To assess the survival-prognostic value of three measures of autonomic function in relation to cardiovascular disease and diabetes. **Materials and Methods:** The study population (n=631) consisted of a glucose tolerance stratified sample from the 50 to 75 year-old general population of the town of Hoorn, the Netherlands. Persons with cardiac damage or those who used drugs known to affect the autonomic nervous system functioning were excluded (n=116). Parameters of cardiac autonomic function were derived from non-invasive blood pressure and RR interval recordings during spontaneous breathing and metronome breathing at six cycles per minute, in supine position. During nine years of follow-up 77 subjects died of whom 34 diabetic. **Results:** The hazard ratios [95% CI] for death from all causes expressed per two standard deviations of the autonomic function parameter:

Parameter	Non-diabetics (n=385)	Diabetics (n=130)	All (n=515)
SDNN (ms)	1.06 [0.51-1.75]	2.34 [0.80-6.67]	1.24 [0.71-2.13]
EI diff (ms)	0.98 [0.47-1.79]	4.42 [1.23-16.67] *	1.56 [0.85-2.86]
BRS (ms/mmHg)	1.06 [0.51-2.22]	2.35 [0.87-6.25]	1.49 [0.81-2.70]

** $p < 0.05$ after adjustment for age, gender, impaired glucose tolerance (for 'non-diabetics'), newly diagnosed DM (for 'diabetics'), glucose tolerance groups according to WHO criteria (for 'all'), history of cardiovascular disease, and hypertension; **SDNN** standard deviation of all normal RR intervals; **EI diff** mean expiration-inspiration difference in RR intervals over six consecutive breaths; **BRS** baroreflex sensitivity*

Conclusions: Diminished autonomic function, and EI difference in particular, is associated with increased risk of death from all causes, especially in diabetic patients. This suggests that suboptimal autonomic function may be especially hazardous in diabetic patients.

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Insulin treatment may preserve cardiac autonomic nerve function in diabetic patients

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AIMS: Symptomatic diabetic autonomic neuropathy is one of the disable disorders and may lead to sudden death. However, whether modality of treatment may affect its natural history is not fully evaluated. To delineate long-term decline of cardiac autonomic nerve function, power spectral analysis of heart rate variability was done to depict low-frequency component (0.05-0.1Hz; LF) as a sympathetic nerve function and a high-frequency component (0.2-0.35Hz; HF) as a parasympathetic nerve function. **SUBJECTS and METHODS:** After baseline clinical characteristics, power spectral analysis of 24 hours heart rate variability were evaluated on 19 patients (age 55 ± 9 years, 6 female and 13 male, duration 16 ± 7 years) before and after follow-up period for 5 to 9 years. Eleven patients treated with insulin were divided into two groups according to the state of glycemic control. **RESULTS:** LF showed a marked reduction, although HF in insulin-treated patients did not significantly change. Among insulin-treated 11 diabetics, subjects with a good glycemic control (average HbA_{1C} during the follow-up period < 8 %) showed a significantly smaller change in $\triangle$LF/LF compared with subjects with a poor glycemic control (-3.9 ± 4.6%/year vs. -9.2 ± 2.5%/year, $p<0.05$). Eight patients treated with oral hypoglycemic agents showed a bigger reduction in $\triangle$LF/LF and $\triangle$HF/HF than patients with insulin therapy. **CONCLUSIONS:** Insulin treatment may be protective from cardiac parasympathetic nerve dysfunction as compared to the treatment with oral hypoglycemic agents.

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EFFECT OF TOLRESTAT ON CIRCADIAN VARIATION OF HEART RATE IN PATIENTS WITH DIABETIC AUTONOMIC NEUROPATHY

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Purpose: Evaluation of the effect of tolrestat, an aldose reductase inhibitor, on heart rate variability **[HRV]**, a reliable index of sympathovagal interaction, in 8-hour time intervals, given the paradoxical dominance of sympathetic over vagal tone during the night hours in patients **[pts]** with diabetic autonomic neuropathy **[DAN].** The effect of aldose reductase inhibitors on the circadian pattern of autonomic function in DAN pts has not been studied. **Patients - methods:** 45 pts with DAN [mean age 52 years], 22 IDDM, 23 NIDDM, were randomized into tolrestat [n=22, 200 mgr./day] or placebo [n=23].Parameters of HRV frequency domain were assessed in 8-hour time intervals at baseline and after one year of treatment with a digital Holter monitor. 20 pts with diabetes without DAN and 20 normal subjects were used as controls. **Results:** In comparison to placebo, tolrestat improved significantly [p<0.05] all HRV frequency domain indices during the entire 24-hour period. During the night 8-hour period 23.00- 7.00, time at which prevalence of sympathetic tone was most markedly increased, tolrestat increased high frequency power [vagal], decreased low frequency power [sympathetic] and their ratio [sympathovagal], p<0.01 for all three counts, in comparison to baseline and placebo. Nevertheless, the low to high frequency power ratio was still higher in DAN pts than controls for the 23.00-7.00 time interval, but in a lesser degree. **Conclusions :** The improvement of sympathovagal interaction by tolrestat in DAN pts at time interval most adversely affected might be beneficial in reducing malignant ventricular arrhythmias and sudden death in these pts.

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RELATIONSHIP OF CARDIOVASCULAR AND GASTROINTESTNAL NEUROPATHY IN DIABETICS

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Aims: 1. assess the presence of autonomic neuropathy of the cardiovascular and the gastrointestinal systems and their bilateral relationship in diabetics, and 2. assess the relationship of diagnosed autonomic neuropathy and subjective symptoms, which are common in the affected population. These symptoms include the following: cardiovascular, gastrointestinal, genitourinary, sudomotor system, and the syndrome of unawareness of hypoglycemia. **Methods:** The study group was comprised of 25 type I diabetic patients (12 women and 13 men), with a mean age of 40.5 ± 11.6 (range 21 to 57 years), having a mean duration of diabetes of 17.8 ± 7.9 (range 4 to 35 years), and treated with an intensive insulin regimen. The cardiovascular autonomic neuropathy was examined by the computerized system VariaPulse TF 3. Scintigraphy was used to investigate the gastric emptying time of 99 m Tc labelled rice. The data regarding the subjective symptoms was gathered from questionnaires. The statistical analysis was conducted using Spearmen Correlations and ANOVA. **Results:** A statistically significant correlation was found between the presence of autonomic neuropathy of the cardiovascular and gastrointestinal systems (r= 0.634 p< 0.0007). A statistically significant correlation was found between cardiovascular and gastrointestinal neuropathy and erectil dysfunction (r= 0.48, p< 0.0078) (r= 0.42, p< 0.0388) and the syndrome of unawareness of hypoglycemia (r= 0.49, p< 0.0057) (r= 0.52, p< 0.0075). There was no statistical significance found between cardiovascular autonomic neuropathy and the subjective symptoms related to the cardiovascular system. There was no statistical significance found between gastrointestinal neuropathy (impaired gastric emptying) and the subjective symptoms of the gastrointestinal system. **Conclusions:** This study suggests that cardiovascular autonomic neuropathy in diabetics may be a warning sign of autonomic neuropathy in other body systems, which are more difficult to diagnose. Additionally, reported subjective symptoms of the diabetic do not correlate with the presence of visceral neuropathy.

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Autonomic Neuropathy – G. I. Tract and Small Fibres

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THE PREVALENCE OF GASTROINTESTINAL SYMPTOMS IN DIABETICS COMPARED TO A GENERAL POPULATION.

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Aims: To compare the prevalence of gastrointestinal (GI) related symptoms in patients with diabetes with a general population. **Materials and Methods:** A patient cohort of 403 type 1 (n= 120, diabetes for a mean of 15,1 years) and type 2 (n= 283, known diabetes for a mean of 7,7 years) diabetics, aged 35-75 years, answered a questionnaire listing a range of GI symptoms. A similar questionnaire was collected in a population study of 2653 men and women aged 40-70 years. **Results:** Frequence in %

SYMPTOMS:	Diabetics	Population
Globulus sensation	30.5	9.2*
Dysphagia	23.6	4.7*
Early fullness	42.8	6.8*
Nausea	30.6	13.0*
Vomiting	21.4	7.6*
Borborygmia	52.5	21.4*
Bloating	57.7	36.4*
Changing consistency of stool	66.2	30.1*
Changing frequency of stool	57.3	14.1*

* p<0,05

Globulus sensation, dysphagia, nausea, vomiting, borborygmia and bloating is significantly correlated with BMI (Weight/height^2) in the patient cohort, but not in the general population. Dysphagia, nausea and vomiting is related to urine albumin excretion, and dysphagia and changing consistency of stool to peripheral neuropathy. There is no significant correlation between HbA1c, duration of diabetes and GI symptoms. **Conclusions:** Compared to the general population diabetics suffer significantly more often from GI symptoms related to overweight, micro/ macro-albuminuria and peripheral neuropathy.

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GASTRIC MYOELECTRICAL ACTIVITY IN DIABETIC TYPE 1 PATIENTS

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Abnormalities in gastric myoelectric activity may be associated with gastric motility disorders. **The aim** of the study was to investigate correlation of gastric myoelectric activity with glicemia measured during the test, metabolic control (HbA1c) and late complications of diabetes. **Material and methods**: Studies were done on group of 70 diabetic type 1 patients to evaluate gastric myoelectric activity. Mean time duration of diabetes was 14.7, mean age of the group was 36.4 years. Gastric myoelectric activity was recorded with use of skin electrodes and Digitraper EGG system - Synectics Co. Mioelectrical activity of stomach was measured in preprandial and postprandial phase. During the hole gastrointestinal testing glicemia was controlled every 30 minutes (G0, G30, G60, G90, G120). Patients with anamnesis of surgical gastrointestinal intervention, gastric or duodenal ulceration, taking medications which could influence gastrointestinal motoric function were excluded from the examination. Cardiovascular neuropathy was estimated using ProSciCard in analysis of cardiac rhythm variability during 5 minutes observation in rest, in tests of deep breathing, vertication and Valsalva test. Peripheral neuropathy and retinopathy was evaluated according to the specialistic examination. Nephropathy was diagnosed depending from the result of microalbuminuria test. **Results**: The group of patients with low and high gastric myoelectric activity [1°pre PDF (period dominant frequency) <2 and post PDF <2, 2°pre PDF >2 and post PDF >2] had significantly higher incidents of late complications of diabetes and gastrointestinal symptoms. In both groups of patients, we observed correlation between glicemia G60, G90 and frequency of gastric myoelectric activity in preprandial phase (p< 0.05). In group of 19 patients with pre PDF> 2 and post PDF <2 there was correlation between G30,G60,G90,G120 and preprandial myoelectric gastric activity. (p< 0.05; r= 0.66).There was also significant correlation between HbA1c and frequency of gastric myoelectric activity in preprandial and postprandial phase (p< 0.05 ; r =0.94 , r=0.51). **Conclusion**: The data in these study suggest that there is an association between glicemia, HbA1c and gastromyoelectic activity.

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DIABETIC GASTROPARESIS AND DOPAMINE DA_2 RECEPTORS

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Aims: hyperglycaemia increases the dopamine (DA) receptors activity. As dopamine has inhibitory effects on gastric emptying (GE), diabetic gastroparesis (DG) could be related to increased dopaminergic activity in myenteric plexus. If so, antidopaminergic drugs may be more useful than other prokinetic agents in treating DG. To verify this hypothesis we compared the effect of levosulpiride (L), a selective dopamine DA_2 receptors antagonist, to cisapride (C), a gastrokinetic drug devoid of antidopaminergic properties. **Materials and Methods**: we studied 16 non diabetic patients (9 F and 7 M, age: 43±3 years) with functional gastroparesis (FG) and 15 type 1 diabetic subjects (6 F and 9 M, age: 45±2 years and duration of diabetes: 16±1 years) with DG. GE was investigated by the ^{13}C-octanoic acid breath test. Meal test (one egg, two slices of white bread, 5 g of margarine and 150 ml of water) was ingested within 10 min after an overnight fast; 100 µg ^{13}C-octanoic acid (Cortex Italia, Milan, Italy) was incorporated into egg yolk. Breath samples were collected just before, immediately after finishing the meal (t=0) and every 15 min for 4 hours. Delayed GE was defined as gastric emptying coefficient (GEC) < 2.6 (corresponding to the 95% confidence interval in our group of 30 healthy volunteers). Having ascertained gastroparesis by measuring GE in basal condition, all patients received randomly and according to a cross-over design 2 doses of L (25 mg) or C (10 mg). L and C were administered per os at 10 p.m. on the day before the test and 30 min before the test meal (at least 4 days elapsed between each test). Δ GEC was calculated as the difference between the basal value and the value after treatment with L and C. The results were compared by Student's t-test. **Results**: in diabetic patients L fastened GE more than C (Δ GEC = 1.29 ± 0.73 vs 0.53 ± 0.23, $p < 0.05$). The gastrokinetic effect of L was significantly higher in DG than in FG (Δ GEC = 1.29±0.73 vs 0.63±0.41, $p < 0.05$) On the contrary C improved GE in the same way in DG as FG (Δ GEC = 0.57 ± 0.23 vs 0,68 ± 0.31, p=ns). **Conclusions**: the antagonist of dopamine DA_2 receptors L is more efficient in fastening GE in DG than in FG. These data support the hypothesis that increased dopaminergic DA_2 receptor activity represents an important mechanism in delaying GE in diabetes.

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EVAULATION OF DIABETIC NEUROPATHY IN PATIENTS WITH MULTIPLE-SITE GASTROINTESTINAL HYPOMOTOLITY

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Aims: This study was designed to analyze the characteristics of the dysfunction in different digestive organs in patients (pts) with long-standing type-1 diabetes mellitus (DM) and to evaluate the influence of neuropathy on gastrointestinal motility.
Materials and methods: 10 pts with type-1 DM of long duration (age: 44.3±6.8 years [yrs], duration: 26.3±3.9 yrs, BMI: 24.1±2.9 yrs, mean±SE) were included. 6 healthy subjects were tested as controls. The emptying of the esophagus, stomach and gallbladder (GB) was evaluated by scintigraphic methods. Autonomic neuropathy (AN) was assessed by means of conventional cardiovascular reflex tests. Sensory nerve function was studied with a Neurometer (Neurotron Inc. Baltimore, Md), using transcutaneous electrical stimulation.
Results: The esophageal emptying was impaired in the DM pts in comparison with the normal range for the non-DM subjects (mean percentage of emptying in DM pts: 63.3±8.4%, normal range: >90%). The hypomotility of both the stomach and the GB was proven by the scintigraphic tests (half-time of gastric emptying [HTE]: 105.2±17.6 *vs* 54.2±15.4 min., $p<0.05$, GB ejection fraction [GBEF]: 38.5%±7 *vs* 78.3±5.8%, $p<0.01$, DM *vs* controls). A positive correlation was observed between HTE and the AN score ($r=0.65$, $p<0.05$), while the correlation was negative between GBEF and the AN score ($r=-0.66$, $p<0.05$). There was a negative correlation between HTE and the Valsalva manoeuvre ($r=-0.84$, $p<0.001$), and HTE and the heart rate response to breathing ($r=-0.68$, $p<0.05$). A positive correlation was proven between GBEF and the Valsalva manoeuvre ($r=0.72$, $p<0.05$), and GBEF and the heart rate response to breathing ($r=0.71$, $p<0.05$). There was not a significant relationship between the esophageal emptying and the AN parameters. The assessment of the peripheral nerve function demonstrated high mean perception in the lower limb (current perception thresholds at three frequencies on the peroneal nerve: 5.54±0.8, 3.94±1.1, 2.51.±0.9 mA).
Conclusions: Delayed esophageal, gastric and gallbladder emptying are all characteristic features of long-standing diabetes mellitus. This study underlines the important role of neuropathy in the pathogenesis of gastric and gallbladder hypomotility.

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CLINICAL ASPECT OF THE MEASUREMENT OF SYMPATHETIC SKIN RESPONSE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Sympathetic skin response (SSR) induced by an electric stimulation is an useful marker for reflecting the function of the descending tract of the sympathetic pathway. The aim of the current study was to clarify whether SSR would be associated with the pathogenesis of diabetes mellitus and/or with the progression of diabetic complications. Studies were performed in 40 type 2 diabetic patients (23 males, 17 females, mean age, 60.1 y-o) and 10 age-matched healthy subjects. SSR was measured using an electro-myogram in the right hand. We evaluated the amplitude (SSR-A) and the latency (SSR-L) after an electric stimulation, as a nerve function. Thirteen of 40 (33%) diabetic patients could not detect a rectangular wave. Diabetic patients showed significantly lower SSR-A and remarkably longer SSR-L compared with healthy subjects. Eleven of 27 (41%) patients revealed a reduced SSR-A level. In diabetic patients, SSR-A exhibited a significantly reverse correlation with the diabetes duration. MNCV showd a significantly positive correlation with SSR-A and did a negative correlation with SSR-L. SNCV also showd a significantly negative correlation with SSR-L. The descending values of sBP in Shellong's test tended to be positively correlated with SSR-A. R-R time interval variation on ECG presented a significantly positive correlation with SSR-A. SSR-A showed a significantly reverse correlation with urinary albumin excretion.
Conclusions: The present data suggest that both SSR-A and SSR-L are clinically useful tools to reflect the sympathetic nerve function, and are influenced by the diabetes duration and the progression of nephropathy.

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THE PREVALENCE OF ERECTILE DYSFUNCTION IN A MALE DIABETIC POPULATION.

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Aims: To determine the prevalence of erectile dysfunction (ED) and relation to exposure in a male diabetic population. **Materials and methods:** A patient cohort of 326 males (Type 1 diabetics: n= 130; mean age 46,1, SD 16,3 years and Type 2 diabetics: n= 196; mean age 61,1, SD 15,6 years) answered a questionnaire about ED, diabetes regulation, concomitant illnesses and psychical vulnerability. **Results:** For men ≤ 40 years (n=64) 7,8% had problems obtaining a satisfactory erection. For men > 40 years 63,0 % of type 1 diabetics (n=76, mean age 57,4, SD 11,6 years) and 71,7 % of type 2 diabetics (n= 186, mean age 62,4, SD 10,7 years) were suffering from ED. Logistic regression, after stepwise exclusion, revealed the following independent risk factors for ED:

Variable	Odds Ratio	95% CI
Age 41-55 >< ≤ 40 years	7,39	2,17 – 25,19
Age 56-65 >< ≤ 40 years	13,32	3,53 – 50,25
Age 66-75 >< ≤ 40 years	11,20	2,61 – 48,08
Reduced >< normal vibration sense	1,63	0,68 – 3,91
Ceased >< normal vibration sense	3,19	1,25 – 8,19
Hypertension	2,30	1,12 – 4,69
History of pulmonary stasis (CVD)	5,64	1,21 – 26,32
Psychical vulnerability	2,66	1,06 – 6,67

Conclusions: our study revealed a very high prevalence of ED. Only 11% of the patients were examined and 10 % treated for their problem. The strongest independent risk factor for ED was age, but also peripheral neuropathy, hypertension, CVD and psychical vulnerability were significant risk factors.

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Smaller fibre neuropathy in the pathogenesis of Diabetic Erectile Dysfunction.

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Erectile dysfunction (ED) in diabetes is thought to be caused by both neuropathy and vasculopathy of diabetes. However, very little is known about the exact roles they play in the genesis of ED. Aims: To compare the neurological and vascular function in diabetic patients with ED to those without ED. Materials and Methods: 18 diabetic patients with ED and 25 diabetic control subjects underwent peroneal motor and sural sensory nerve conduction, warm temperature discrimination threshold (TDT) in their feet, vibration perception threshold (VPT) on the great toe, standard cardiac autonomic function tests (AFT) and ankle brachial pressure index (ABPI). Patients with ED (except one) also had Pharmaco-Penile Duplex Ultrasonography of cavernous arteries following intracavernosal injection of 20 microgram alprostadil. Results: All patients with ED had normal ABPI (1.22 ± 0.4 SD). However there was severe vascular disease on PPDU (peak systolic velocity < 25cm/sec) in 9 (53%). The mean TDT of the group with ED (9.9°c) was significantly ($p<0.001$) higher compared to the control (I.6 °c). Similarly age corrected VPT and nerve conduction studies were also significantly abnormal (but not as strongly as TDT) in the group with ED. In contrast, there was no difference in AFT between these groups. Conclusion: Contrary to conventional opinion, ABPI or abnormal cardiac AFT were not related to ED, but Pharmaco-Penile Duplex Ultrasonography and TDT were impaired in diabetic ED. This finding suggest that small fibre neuropathy has a significant role in the pathogenesis of diabetic ED.

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AUTONOMIC FUNCTION AND AUTOANTIBODIES TO AUTONOMIC NERVOUS TISSUES AT FOLLOW -UP IN A COHORT OF YOUNG PATIENTS WITH TYPE 1 DIABETES.

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Aims: Recent studies have linked autoimmunity to nervous tissue structures and diabetic autonomic neuropathy. To evaluate the early stage of Type 1 diabetes and the natural history of this association, we re-assessed the autonomic function and the presence of autoantibodies to autonomic nervous tissues in a cohort of diabetic adolescent patients previously recruited (follow-up at 40±3 months). **Material and Methods:** Four standard cardiovascular (CV) tests (using an automated system and age-related index values) and autoantibodies (Ab) to sympathetic and parasympathetic nervous structures (using an indirect immunofluorescent complement-fixation technique, with rabbit cervical ganglia and vagus nerve as substrates) were performed in 74 (mean age 18.1±1.6, mean diabetes duration 10.2±3.5 years) of the 84 (88%) Type 1 diabetic patients and in 17 of the 45 matched controls (38%), previously recruited. **Results:** 7 patients (9%) had one abnormal CV test, and 2 had blood pressure drop >30 mmHg; none had autonomic symptoms. Values of deep breathing (DB) (33.8±8.5), 30:15 (1.43±0.18) and Valsalva ratio (1.8 ±0.3) tests were significantly lower compared to the values at recruitment (37.9±9.4, 1.45±1.15, 2±0.3, respectively), amongst the diabetic patients ($p<0.005$, paired t-test), while in the control groups only values of DB test were lower. Values of each CV test were not significantly different between patients and controls. Nine patients (12%) were positive for anti-vagus nerve Ab, and 12 patients (16%) for anti-cervical ganglia Ab ($p< 0.05$ vs controls). The Ab persisted in the patients positive at recruitment. Ab positive patients, considered together as a single group, scored lower values of CV tests, although not statistically different compared to Ab negative patients and controls. **Conclusions:** Our data indicate that diabetic autonomic neuropathy is not characteristics of young diabetic patients, but that the nervous tissue autoantibodies are present and persist in the first 20 years of disease. Follow-up studies will continue to evaluate future autonomic dysfunction and symptoms and to establish whether the subtle autonomic dysfunction detected and/or the autoantibodies are predictive of the development of this complication.

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LEFT VENTRICULAR HYPERTROPHY AND PERIPHERAL SENSORY NEUROPATHY IN DIABETIC PATIENTS: IS THERE CONNECTION?

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Aims: Diabetic neuropathy is the most common diabetic complication. Myocardial infarction is the most frequent cause of death in patients with type-2 diabetes mellitus and the third one in type-1 diabetic patients. Left ventricular hypertrophy with risk of ischemic heart descase is a well known complication in diabetic patients without hypertension. Trying to find a connection between the most common complication and cause of death in diabetes, we suppose that the damage of the sensory innervation of the heart may play a role in diabetic heart descase. The aim of our study was to investigate the connection between the diabetic peripheral sensory neuropathy and left ventricular hypertrophy as a risk factor of myocardial infarction. **Patients and Methods**: We examined 44 diabetic patients (age: 58.9 ±12.5 ys, duration of diabetes: 10.4±7.2 ys, IDDM: 9, NIDDM: 35, male: 16, female: 28). M-mode echocardiography was performed to measure posterior and septal wall thickness and the left ventricular diastolic diameter to calculate the left ventricular mass index as markers of the left ventricular hypertrophy. Peripheral neuropathy was studied with Neurometer® device (Neuroton Inc., Baltimore, MD) measuring current perception threshold at three different frequences, that gives an opportunity to examine large (5ooo Hz), small (25o Hz) myelinated and unmyelinated (5 Hz) nerve function in a non-invasive way. **Results**: Septal thickness correlates significantly with large myelinated ($p=0.041$) and small myelinated ($p=0.015$) nerve failure. Unmyelinated nerve dysfunction correlates significantly with all the parameters refering to left ventricular hypertrophy, that is posterior wall thickness ($p<0.005$), septal wall thickness ($p<0.005$) and left ventricular mass index ($p<0.0001$). **Conclusion**: Our results seem to confirm our hypothesis, i.e. peripheral sensory neuropathy may play a role in diabetic left ventricular hypertrophy.

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SMALL NERVE FIBRE FUNCTION, NEUROVASCULAR CONTROL AND SUBSTANCE P IN DIABETES MELLITUS TYPE 1

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Aim: Patients with diabetes mellitus are on risk of developing small nerve fibre dysfunction and disturbances in neurovascular control. The 11-amino-acid peptide, substance P is found in C- and Aδ nerve fibres and is involved in the axon-flare response. The present study investigates small nerve fibre function, neurovascular control and plasma substance P in type 1 diabetes patients. **Subjects and methods:** The microvascular response to thermal injury was investigated in 29 type 1 diabetes patients (age: 39.1±11.4 years; duration of diabetes: 13.1±1.8 years; HbA1c: 7.9±0.5 %; mean±SEM) and in 17 healthy control subjects (age 34.4±3.5) using laser doppler flowmetry (LDF, MBF 3D, Moor Instruments, Devon, UK). All subjects were free from peripheral macrovascular disease or foot ulceration and without any vasoactive drugs. Small nerve fibre function was assessed by the investigation of cold, heat and pain perception thresholds (Path Tester, PHYWE, Göttingen, Germany). Substance P plasma levels were measured by a competitive ELISA using purified plasma samples (C-18 reverse phase cartridges). **Results:** The group of diabetic patients with small nerve fibre dysfunction showed a decreased microvascular response following thermal injury compared to the diabetic group without neuropathy and the healthy control group (32.3±19.7 vs. 64.8±9.5 AU, $p<0.01$; vs. 92.8±26.9 AU, $p<0.01$; respectively). Decreased plasma substance P levels were found in patients with a diminished pain perception threshold (3.3±2.0 ng/ml), a diminished warm perception threshold (5.9±2.1 ng/ml) and a diminished cold perception threshold (7.9±1.4 ng/ml) compared with the control group (11.0±1.5 ng/ml $p<0.05$ respectively). No significant difference was found between diabetes patients without neural dysfunction and the control group. No significant difference in plasma substance levels were found with regard to the vibration perception threshold between the different groups. **Conclusions:** Our results confirm a disturbed microvascular function and reduced plasma substance P levels in type 1 diabetic patients with small nerve fibre dysfunction. A diminished release of substance P from damaged small nerve fibres might be involved in microvascular dysfunction in type 1 diabetes mellitus.

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Diabetic Foot – Epidemiology

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DIABETIC FOOT LESIONS AND THEIR TREATMENT IN TIMIS DISTRICT, ROMANIA

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Aims: In view of the St. Vincent Declaration recommendations, to reduce by one half the rate of limb amputations for diabetic gangrene, the present study assessed the number of patients with diabetic foot lesions hospitalized in our Department in two 5 year-time-periods (1986-1990, and 1991-1995), and the percent and site of amputations performed.
Materials and Methods: During the first time period, 88 subjects (1.3% of the total admissions for diabetes) were hospitalized for diabetic foot lesions, while 98 (1.9%) were admitted during the second time-period. Patients mean age was similar in 1986-1990 [62.6 (17.8) years] and in 1991-1995 [64.3 (12.3) years].
Results: Most of these subjects with foot lesions (70.5% in 1986-1991 and 68.4% in 1991-1995) had either peripheral vascular disease or both vascular disease and neuropathy. The rest (29.5% in 1986-1990 and 31.6% in 1991-1995) had neuropathic foot ulcers without ischemia. The limb was preserved in more than one half of these patients, in both time-periods (55.4% and 53.3%, respectively). Limb amputations were necessary in 44.6% of the patients in 1986-1990, and in 46.7% in 1991-1995. Minimal amputations with heel preservation were performed in 23% of the subjects in the first time-period and in 12.2% of them in the second period ($p<0.05$). The percent of below-knee amputations was similar (13.5% and 14.1%) while that of extended interventions (above the knee) increased from 11.1% in 1986-1990 to 17.4% in 1991-1995 ($p=0.01$).
Conclusions: The number of patients with diabetic foot lesions hospitalized in our Department and the percent of limb amputations performed for these lesions did not change significantly over 5 years. The implementation of the St. Vincent Declaration's goals requires a longer period of time and an improvement of cultural, educational and socio-economic background of patients with diabetes.

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DIABETIC FOOT COMPLICATIONS INCIDENCE IN GEORGIA.

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The three year (1996-1998) epidemiological study, under the EuroDiabCare programme, that involved 942 diabetic patients, was conducted in different regions of Georgia. Subjects: 942 patient - 59% female and 41% male, 17% with IDDM and 83% with NIDDM with mean duration of diabetes 11.3±8.9y.Methods: clinical examination according to Basic Information Sheet, examination of vibration sensitivity with tuning fork, pink sensitivity by means of 5.07/10gr monofilament.Results: the study showed that 58.8% were suffered of low vibration sensitivity with a mean age 60.0±11.1y. and duration of diabetes 13.25±8.69y. 20.7% were suffered of low pink sensitivity with the mean age 60.12±11.9y. and mean duration of diabetes 14.39±9.67y. Healed ulcer was revealed in 2.24% of cases with mean age 56.85±14.3y and duration of diabetes 15.0 8.6y. Acute ulcer or gangrene was found in 2.24% of cases with the mean age 59.8±9.68y. and duration of diabetes 15.0±8.6y.Amputations incidence was in 3.5% of cases. We have found amputations prevalence in male patients (5.94%) then in female patients (1.8%). The incidence of amputations during last 12-month period was 1.7%. Conclusions: Despite the implementation of Diabetic Foot Preventive Programme in different Diabetes Centers of Georgia, the frequency of diabetic foot complications is still high, which calls for enlarging of areas of Diabetic Foot Preventive Programme.

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PREVALENCE AND RISK FACTORS OF FOOT LESIONS IN PATIENTS WITH DIABETES MELLITUS IN DNIESTER REGION OF MOLDAVIA

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Aims: The amputations rate in people with diabetes in newly independent countries of the former Soviet Union is high. However, the prevalence of diabetic foot syndrome in these countries remains unknown. The purpose of this study was to investigate the prevalence and risk factors of foot lesions in patients with diabetes in Dniester region of Moldavia. **Materials and Methods:** We examined 320 diabetic patients aged 30-75 years in community-based study and presence of foot ulceration (according to Wagner's classification) was noted. **Results:** We found that foot ulceration I-Y degree was present in 50 (15.6%) patients. The superficial foot ulcers were found in 8 patients (16%), deep ulcers complicated by development of infection were present in 14 patients (28%) and ulcers which subsequently led to gangrena were diagnosed in 28 people (56%). In 12 of those patients (24%) it was nessessary to perform an amputation of the leg and in 30 (60%) of those people the amputation of fingers and part of foot was done. Among patients with foot ulcers there were more male (72%) than female (28%). Most patients with foot ulcers were older than 45 years- 28 people (56%) were 45-61 years old, 12 patients (24%) were 62-70 years old and 6 (12%) were older than 70 years. Most patients had type 2 diabetes (92%). The duration of disease in this cohort of people was the following: newly diagnosed diabetes was in 6 (12%), less that 7 years of duration - in 22(44%), 8-14 years - in 14 (28%) and 15-21 years - in 8 (16%). In 92% of patients the severe neuropathy was present diagnosed using diabetic neuropathy score. Furthermore, all of those patients had poor metabolic control with the levels of HbA_{1c} ranged from 10.2 to 12.6% and only 24% were treated with appropriate insulin regimen. **Conclusions:** Development of foot ulcers and need of amputation are attributed to late diagnosis and poor metabolic control of diabetes mellitus in studied cohort of patients. Improvement of quality of diabetes care could decrease the rate of foot ulcers and amputations in diabetic patients in postcommunist countries of former Soviet Union.

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LOWER EXTREMITY AMPUTATION IN PEOPLE WITH DIABETES, A COMPARISON OF 4 REGIONS IN NORWAY.

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Aim: The St. Vincent Declaration Action Program calls for 50% reduction of amputations in diabetics, a reduction which has been shown achievable in a number of studies. In Norway the data on amputations in diabetics is scarce and we wanted to get a better overview. **Material and methods:** National statistics from the Norwegian institute of hospital research (NIS) from 1993 and 1994 show almost 4-fold variation in non-traumatic amputation incidence between counties. Two counties among those with the highest and two among those with the lowest incidence of amputations were selected. The protocols of the surgical theatres and the electronic patient records and then the records of each patient were examined. **Results:** A total of 299 persons fulfilled the inclusion criteria, 282 were above ankle (major amputations) and 6 patient records were missing. We found nearly the same total number of amputations and the same difference between counties as the data from NIS. Of the 293, 120(41.0%) were diagnosed as having diabetes at the time of surgery. At county level the % was 28.6(AA), 42.7(R), 42.7(NT), 46.7(T). The incidence (n/100.000pop) of amputations in the 4 counties were for AA 27.2(total) and 7.1(diabetes), R 16.8 and 7.1, NT 32.1 and 13.7 and for T 15.1 and 7.0. More diabetics than non-diabetics were amputated below knee, had two or more amputations and were more often amputated bilateral. 80% or more of the diabetics were probably type 2. In 132(46%) patients amputation had been preceded by vascular surgery, AA 26%, R 52%, NT 39%, T 64%. 36% were still smoking and 21% had quit smoking more than 1 year before the first amputation. 24% of the diabetics, 44% of the non-diabetics, 19%of women and 48% of men were still smoking. **Discussion/conclusion**: The data from NIS on total number of amputations seems to be reliable. The reason for the difference between counties is unclear. We do not know if there is a difference in indication for amputation. The prevalence of diabetes and proportion of old people may be different. The numbers offered vascular surgery seems to differ. Further investigation is needed to explain the difference and take action to reduce the number of amputations.

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INCIDENCE OF LOWER EXTREMITY AMPUTATIONS IN THE KRAKOW REGION IN POLAND.

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Aims: The aim of the study was to estimate the incidence of lower extremity amputations (LEAs) in diabetics in Poland – first, background data in this population. **Materials and Methods**: This was a cross-sectional study of the incidence of non-traumatic and non-neoplastic LEAs in the population of Krakow region (1,239,703 inhabitants) in 1996 (it was calculated that for this population size it was sufficient to collect data for one year). Amputations were identified in two sources: thirteen surgical wards (patients' case records and operation room records) and one limb fitting centre. Incidence rates were standardised for the European population. Diabetic specific incidence was calculated using a 1986 survey of diabetes prevalence. **Results**: 290 LEAs were identified. Using capture-recapture method it was estimated that over 90% of cases had been found. Total standardised incidence rate (per 100.000 of total population) was 25.1 (95%CI 22.1-28.1), in non-diabetics 13.2 (95%CI 11.1-15.4), in diabetics 11.9 (95%CI 9.8-13.9), and primary amputations in diabetics 10.4 (95%CI 8.5-12.3). Estimated incidence of LEAs per 100.000 diabetics was 194.9, 313.6 in males and 76.3 in females. In all groups amputations were significantly more frequent in men. Females with diabetes were more susceptible to LEA than non diabetic ($p=0.03$). Minor amputations were more frequent in diabetics than in non-diabetics (31.7 vs 13.3% respectively, $p<0.001$). There was a significant discrepancy of the proportion of minor amputation between surgical wards ranging from 9.23% to 19.5%, which could not have been explained by differences in case mix. **Conclusions**: Incidence of LEAs was found lower than expected. However, Krakow region is an urban area with a large university hospital, therefore further research in other parts of Poland is necessary. Almost 50% of amputations was performed in diabetics, so an improved diabetic foot and gangrene prevention programme should have a considerable impact on the total incidence of amputations. A large differences in the proportion of minor amputations between surgical wards suggests the need for standardisation of the surgical approach towards amputations in diabetics.

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DIABETIC FOOT ULCERS – EPIDEMIOLOGY AND CORRELATION WITH SOCIAL CLASSES AND SELFCARE.

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Aims: To determine the correlation between social classes and knowledge about self-care and diabetic foot ulcers.

Material and methods: A cohort of 468 diabetic patients, 170 type 1 & 298 type 2 was included. There were 277 males and 191 females, mean age 57.2 years range (17-88). All patients answered a questionnaire about self-care, education and former events of amputations and ulcers. All patients had a foot examination at the same time as the questionnaire. The patients were divided into 4 social classes according to their length of school and education.

Results: 24 patients (5.1 %) were amputated, 2 had major amputations (below knee). 112 (23.1%) patients had a previous foot ulcer. 30 patients (6.4%) had an ongoing foot ulcer, and 22 (73,8%) of these had a previous ulcer history. There was no correlation between social class and previous or ongoing ulcer. Patients with a previous ulcer had a significant better knowledge about self-care. 83 % of the patients with previous foot ulcers had significant reduced sensation on the foot evaluated by biothesiometri, compared to 55,4% in the non-ulcer group. Foot ulcers were significantly more common in patients with high BMI .

Conclusions: Our study found no correlation between social class and history of foot ulcer. Patients with a previous history of foot ulcer had a significant better knowledge about self-care. Despite this 73.8 % of the patients with ongoing ulcers had a previous ulcer. This indicates that improved knowledge doesn't necessarily change lifestyle.

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PROSPECTIVE DOCUMENTATION OF AMPUTATIONS IN NORTHRHINE

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Aims: In Germany only scarce data is available from retrospective studies concerning the incidence of amputations. In this study amputations are documented prospectively by surgical departments in Northrhine (9.7 million inhabitants). Methods: In addition, information about preoperative diagnostic evaluation, diabetes status, revascularisation procedures, amputation level, the fate of the patient after amputation, etc. is documented. These data will be used for continuous quality control. Results: The program started in fall 1997 and 93 surgical departments out of 193 in Northrhine participated until December 1998. A total of 1371 amputations in 1049 patients were recorded. 72% of the patients had diabetes (median age 71 years). Infection/sepsis was the cause for minor amputation (below ankle) in 42% of the diabetic patients (D) as compared to 19% in the non-diabetic patients (ND). Major amputations in D were due to infection in 24%, to arterial vascular disease in 21%, and to the combination of infection and arterial vascular disease in 53% (ND 6%, 38%, and 52%, respectively). Initial minor amputations were more common in D with 62% vs. 37% in ND. However, during the hospital stay 13% of the D and 5% of the ND had to be re-amputated at a level above the ankle. Therefore, at the end of the hospital stay (mean duration 33 days), 51% of the D and 66% of the ND had a major amputation above the ankle. Only 44% of the D and 46% of the ND went home after a major amputation. In this group 13% of the D and 7% of the ND died during the hospital stay, 11% (D) and 12% (ND) were transferred to a nursing home, 32% (D) and 34% (ND) had to be transferred to another hospital for further treatment mostly due to the major amputation. Conclusion: This complex amputation registry will give not only information about the German situation concerning amputations on a population basis, but will also allow a continuous quality control to identify the necessary improvements in the care of the diabetic-foot-syndrome.

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ETHNIC DIFFERENCES IN RISK AND DETERMINANTS OF LOWER LIMB AMPUTATION IN PEOPLE WITH TYPE 2 DIABETES.

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Aims: (i)To determine the difference in type 2 diabetes related amputation rates in African Caribbeans compared with Europeans in the UK (ii) to examine the reasons for any difference detected. **Materials and methods:** All cases of diabetes related amputation performed in SE London between 1992-1997 were identified and data on risk factors abstracted from case notes. For each amputee data on two patients with diabetes who had not had amputation (controls) were abstracted from primary care sources. **Results:** First diabetes related amputations were identified in 61 Europeans, and 21 African Caribbeans. Amputation rates, age standardised to the general population, were 13/100 000 in Europeans and 48/100 000 in African Caribbeans (RR 3.7, 95% CI 2.2, 6.1, $p<0.001$). However rates standardised to the diabetic population were 241/100,000 and 224/100,000 in Europeans and African Caribbeans respectively (RR 0.9, 95% CI 0.5, 1.9, p=ns). The ratio of African Caribbeans to Europeans was identical in cases and controls (odds ratio (OR) 1.0, 95% CI 0.5,1.8), but there was a striking gender difference in risk of first amputation. In men, African Caribbeans had a lower risk of amputation than Europeans, (OR 0.5, 95% CI 0.2, 1.3, p= 0.2), in women, this risk was higher (OR 2.8, 95% CI 1, 8, $p<0.04$). Protective factors in African Caribbeans men included a lower risk of previous ischaemic heart disease (IHD) (9% v 36% in controls, $p=0.02$). When this was adjusted for, there was no longer an ethnic difference in risk of amputation (OR 1.1). In contrast, for women, African Caribbeans had equivalent rates of IHD to Europeans (26% v 24%), and marginally higher rates of neuropathy, peripheral vascular disease, previous leg ulcer and retinopathy. But adjusting for these risk factors only partially attenuated the OR (2.1). **Conclusions:** African Caribbeans men have lower rates of diabetes related amputations, whilst African Caribbeans women have higher rates. The ethnic difference in men can be accounted for by differences in macrovascular disease. In women it is likely that ethnic differences in several complications play a role.

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EPIDEMIOLOGY OF LOWER EXTREMITY AMPUTATIONS AND PROPORTION ASSOCIATED WITH DIABETES IN CENTRES IN EUROPE, NORTH AMERICA, AND EAST ASIA

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Aims: To compare the incidence of, and distribution by age and sex of lower extremity amputations and the proportion associated with diabetes in different centres around the world between July 1995 and June 1997.
Materials and methods: 10 centres, all with populations greater than 200,000, in Japan, Taiwan, Spain, Italy, the United States and England provided the data presented here. Cases were identified from at least two data sources (to allow checks on ascertainment); data were abstracted from case notes; denominator populations were based on census figures.
Results: Marked differences between the centres existed in the incidence of amputations, with the highest rates in the Navajo population (e.g. 43.9/100,000/ yr for first major amputations in men) and the lowest in Tochigi, Japan (e.g. 3.8/100,000/yr). In every centre the incidence of amputation rose steeply with age, with most amputations occurring over the age of 60(>60% in 9 of the 10 centres); in most centres the incidence was higher in men than women (from 17% higher for first major amputations in Leicester, England, to 400% higher for all major and minor amputation in Madrid, Spain); and in most centres the incidence of major was greater than that of minor. The proportion of amputations associated with diabetes varied greatly between the centres: e.g. from 30% for major and minor in women in Newcastle, to over 50% in Montgomery County USA, to over 80% in the Navajo population.
Conclusions: In all centres diabetes is disproportionately associated with amputation. However, apart from the Navajo population, differences in the known prevalence of diabetes between the centres can not account for the differences in overall incidence amputation. Differences in the prevalence of peripheral vascular disease are likely to be important but this and the role of other factors requires further investigation.

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SUSTAINED AND INCREASED PLANTAR PRESSURES DURING NORMAL GAIT IN DIABETIC NEUROPATHY

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The aim of this study was to investigate dynamic plantar pressures and time parameters in diabetic neuropathic patients with and without a history of plantar ulceration. We compared 93 patients with neuropathy (17 patients with previous ulceration), with 39 age- and weight-matched non-neuropathic diabetic subjects. Neuropathic patients had longer duration of diabetes and worse diabetic control compared to non-neuropaths. A further group of 35 healthy non-diabetic volunteers served as control subjects. Peak plantar pressure (kPa), contact time (ms) at the site of highest pressure and time to peak plantar pressure (ms) were taken from 6 footprints using the Musgrave Footprint^{R} forceplate system. Patients were free walking at their normal pace. Peak plantar pressures (mean $\pm$ SEM) were significantly higher in the diabetic neuropaths compared with healthy controls (806 $\pm$ 26 vs 572 $\pm$ 36; $p < 0.01$); the subgroup with previous plantar ulceration had the highest peak pressures (1059.5 $\pm$ 55). Peak pressures in the non-neuropaths were not significantly different from healthy controls. The highest pressures occurred over the metatarsal heads or the heel. Time to peak pressure was similar in neuropaths and non-neuropaths and no different from controls. Duration of peak pressure (contact time) was significantly increased in the diabetic groups compared to controls (733 $\pm$ 19 vs 633 $\pm$ 36; $p < 0.01$) but not when compared between diabetic groups. The mean contact time in neuropaths represented 78.8 % of stance phase. These results indicate that neuropathic patients have higher and more sustained plantar pressures compared to non-neuropaths. Further work is in progress to establish the significance of these parameters as risk factors to predict future ulceration.

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CAN A PLANTAR PRESSURE MODEL ACCURATELY IDENTIFY LOCATION OF NEUROPATHIC ULCERATION?

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Aim: The purpose of study was to identify pressure measure reports that were useful in identifying the location of known ulceration on the diabetic forefoot.
Methods: Mid-gait walking steps from thirty-six subjects diagnosed with diabetes, neuropathy and ulceration to the plantar forefoot were analyzed using the Pedar in-shoe pressure analysis system.
Results: The time that weight was loaded on the hallux (P=0.034) and lesser digits (P=0.018) was found to be significantly shorter on the feet with ulceration compared to those without. A combination of five pressure measure reports could significantly differentiate between the three common locations of forefoot ulceration. The significant pressure measure reports were the pressure-time integral measured over the whole foot (P=0.003), pressure-time integral (P=0.002 & 0.006)) and peak pressure (P=0.001 & 0.0.024) measured separately at the first and combined lesser metatarsal-phalangeal joint regions respectively. A preliminary model was developed using discriminate analysis that was accurate to 72.7 percent in identifying the location of the subjects' ulceration. Sensitivity and specificity formulas were calculated to demonstrate the diagnostic value of the model. The model accurately identified ulceration locations to a sensitivity of 83 percent and a specificity of 69 percent.
Conclusions: The model is currently under further development in the hope that it will become a useful screening tool for the early identification of patients at risk of plantar foot ulceration. The implication of an accurate screening tool is that it may be a step toward assisting in the prevention and early treatment of ulceration and hopefully a reduction in the number of ulceration leading to amputation.

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ASSESSMENT OF PEAK PRESSURES ON DIFFERENT PLANTAR SITES IN DIABETIC PATIENTS.

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Foot deformities and increased plantar pressure are considered to be one of the main risk factors for diabetic foot ulcers.The aim of this study was to analyse if plantar pressure in different plantart points differ between diabetic and healthy patients. Materials and methods. 57 IDDM and NIDDM patients and 20 healthy subjects (C) aged and sex matched took part in this study. All diabetic patients had ancle/brachial index ≥ 0.8 and had no history of diabetic foot ulcers. NDS was used to diagnose and quantify peripheral neuropathy. EMED system was used to estimate dynamic plantar pressure in different plantar sites: heel (H), big toe (BT), under the first metatarsal (1M), between the second and third metatarsals (2 M), between the fourth and fifth metatarsals (4M). All diabetic patients was divided into the two groups: the group with mild neuropathy (NDS <15, N, n=25) and the group with severe neuropathy (NDS≥15, SN, n=32). The comparison was performed between N, SN and C groups. Results. The peak plantar pressure on the heel (21.6±8.29 vs 21.6± 5.2 vs 22.6±6.1, ns ;M±SD), 2M (27.94 ±12..57 vs 31.8± 18.13 vs 27.00±13.2; ns), and 3M (19.19± 13.07 vs 19.12± 10.27 vs 18.07± 11.06) does not differ between three groups. The peak plantar pressure on the 1Mwas the same between N and SN groups (18.32±11.74 vs 19.32 ± 11.72; respectively; ns) but differ from the control group (15.64 ± 8.31, $p<0.05$). The mean peak pressure on BT was significantly higher on the SN group as compared to N and C groups (32.68 ± 12.28 vs 16.91±5.11 vs 17.91± 6.34, $p < 0.05$).

Conclusion. The findings suggest that peripheral neuropathy)mild or severe) significantly increase the peak plantar pressure under the first metatarsal and big toe which may explain the high frequency of neuropathic ulcers on this sites of the foot.

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DO ROCKER SOLES REDUCE PLANTAR PRESSURE IN PERSONS AT RISK FOR DIABETIC NEUROPATHIC ULCERATION?

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Aim: To evaluate alterations in pressure reduction and gait parameters associated with various outer sole modifications on prescription shoe gear for high risk patients with diabetes.

Methods: Mid-gait walking steps from thirty subjects diagnosed with diabetes, neuropathy and plantar ulceration to the hallux or 1st metatarsal-phalangeal region were analyzed using the Pedar in-shoe pressure analysis system. All subjects were fitted with shoes that had had the outsole modified according to the measures of their metatarsal parabola taken from dorsal plantar radiographs. Two base line shoes were tested, a canvas oxford and an unmodified therapeutic shoe. Five outsoles were tested and they were a fifteen, twenty and twenty-five degree forefoot rocker with the apex placed proximal to the metatarsal phalangeal joint parabola and two twenty degree rockers that were placed level with and distal to the parabola.

Results: Analysis of the means of the peak pressure results are statistically significant ($P<0.05$) but are not clinically relevant due to the significant difference being less than 10kPa difference per rocker shoe. The measured effect on pressures from the different rockers varied per location on the foot and in the presence of ulceration. Of most note was that there were marked differences between individual subjects. Further analysis of the patterns of pressures over time is currently underway. Contrary to current prescribing practice the patterns suggested that the pressure that is experienced by the foot at foot flat far exceeded the pressure at the push off phase of gait.

Conclusions: The prescription of a rocker soled shoe is favorable over a standard therapeutic and a canvas oxford shoe. However, the wide range of individual subject differences suggest that in-shoe pressure assessment may be beneficial to determine which rocker sole is required for each patient.

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A PILOT STUDY OF IN-SHOE TEMPERATURE ASSESSMENT DURING DAILY ACTIVITIES IN HEALTHY CONTROLS AND DIABETIC PATIENTS.

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Previous evidence suggests that diabetic neuropathic patients have elevated plantar surface skin temperature and that this might predict subsequent foot ulceration. However no study has assessed dynamic in-shoe temperatures during daily activities. **Aims:** We therefore initiated a pilot study in 4 controls (C), 5 diabetic controls (DC), 7 diabetic neuropathic patients (DN); of whom 4 with a history of ulceration (DNU). **Methods:** Patients with peripheral vascular disease, on vasoactive medications or smokers were excluded. A temperature sensor was put in the heel area of a PPT insole, which was placed in one of the subjects' shoes. Measurements were made between 4 and 6 hours on three normal days and subjects kept a diary of their activities. **Results:** There were no significant differences in age or duration of diabetes between the studied groups. The average (± SD) Neuropathy Disability Score was higher in DN (6.0 ± 1.0) and DNU (9.5 ± 1.0) compared to Controls (0.0 ± 0.0) and DC (1.2 ± 1.8) ($p<0.05$). The average (± SD) temperature (°C) inside the shoe was higher in Controls (33.6 ± 0.90) compared to DC (29.7 ± 1.0), DN (29.7 ± 1.0) and DNU (31.5 ± 1.1) ($p<0.05$). The variation was higher in DN (1.50) compared to Controls (0.93), DC (1.19) and DN (1.01) and the difference between the average and the 5th percentile was respectively 1.48, 1.54, 1.43 and 1.77 (°C) for C, DC, DN and DNU (ns), indicating a much greater variation around the mean for DNU. **Conclusions:** The results show a trend to increased in-shoe temperature variability in the DNU group, but significantly lower temperature in diabetic patients versus controls. Thus, earlier data of increased foot skin temperatures in diabetic neuropathic patients were not confirmed, suggesting that in-shoe thermoregulation does not follow the same pattern as thermoregulatory responses of the foot exposed to open air. Whether a higher in-shoe temperature in the DNU group can be considered as a risk factor of foot ulceration needs to be explored in a more in-depth study into in-shoe thermoregulatory responses in diabetic neuropathy.

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LASER DOPPLER FLOWMETRY IN THE STUDY OF FEET SKIN MICROCIRCULATION IN IDDM

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Aims. Diabetic foot syndrome arises as the expression of many functional and structural disturbances. The effective prevention is based on defining these disturbances; particularly in microcirculation of the skin of the feet (ulcers). The promissive method for such diagnostics is Laser Doppler. Due to this fact the study was undertaken with Laser Doppler Flowmeter - Perimed cooperating with the IBM computer and special programme. **Methods.** For comparative assessment of the influence of IDDM on feet skin microcirculation 4 groups were examined: 1) 26 healthy subjects, 2) 20 patients with IDDM since 6 months, 3) 25 patients with IDDM between 0.5 to 5 years and 4) 24 patients with IDDM for more than 10 years. All persons were subjected to the following studies: resting flowmetry, occlusive, orthostatic and termoregulatory tests. Flow values were expressed in perfusion units (PU). **Results.** No statistically differences in the flow during test were found in groups 1, 2 and 3; in group 4 the resting flow was decreased (12.7 ± 6.7 PU vs 9.4 ± 3.3 PU). All 3 functional tests revealed the statistically significant impairment of the microcirculation in the adaptation to the testing stimuli in all diabetic groups - 2, 3 and 4. The most striking adaptive limitation was found in IDDM of long duration. **Conclusions.** Laser Doppler flowmetry reveals functional abnormalities of the circulation of the feet skin in IDDM without clinical symptoms of foot syndrome. Intensity of disturbances is related to the IDDM duration. Its application may facilitate finding the patients at risk of developing foot ulcers and syndrome.

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SYNCHRONE AND ASYNCHRONE VIDEO-CONSULTATION TO SUPPORT COOPERATIVE TREATMENT OF THE DIABETIC FOOT

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The treatment of diabetic patients requires interdisciplinary care due to diabetes-associated complications. This applies especially to the diabetic foot syndrome, where a close cooperation between the diabetologist, the surgeon, the interventional radiologist and the specialised shoemaker is necessary. In outpatient settings, difficulties occur because the involved specialists are rarely available at the same place and the same time. Consequently, patients are often bothered with unnecessary transports and waiting time. **Aims:** To solve these problems, a video-consultation-system has been tested in 1997 and has being integrated into routine care in 1998. **Materials and Methods:** The continuous evaluation focused on the video quality, concerning sufficient support for diagnosis and therapy decisions and how much information has to be transferred. Finally, the system`s user-friendliness and the required time per patient has been evaluated. An ISDN-based video-consultation-system for synchronous communication between the diabetic foot care unit and the surgical outpatient clinic has been installed. Additional patient findings (x-ray images) have been transferred using a special document camera. 31 patients were included for evaluation in 1998. **Results:** The quality of the transferred images (video and x-ray) has been judged as sufficient by the surgeon. The system is easy to handle by offering a convenient touch pad interface. During video-consultation, the direct communication between the two parties, discussing relevant patient data, is very important. The mean consultation time was 20 minutes per patient. For 70% of the patients, an instant reasonable and effective therapy decision could be taken. 30% of the patients required transferral to the surgeon for detailed examination. **Conclusion:** Altogether the tested video-consultation-system has been judged as positive and helpful. Some of the problems, mentioned above, could be solved with an asynchronous tele-consultation-tool (electronic mail). In addition the system has to be implemented in other specialities like radiology and specialised shoemakers.

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PROGNOSTIC VALUE OF SOME FACTORS FOR HEALING NEIROISCHEMIC DIABETIC FOOT ULCERS.

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Healing of neiroischemic diabetic foot ulcers is influenced by multiply factors and needs a multifactorial approach. The aim of this study was to investigate the role of some factors in healing process of neiroischemic foot ulcers. Materials and Methods. 26 NIDDM (16 males) patients with neiroischemic foot ulcers (ankle/brachial index < 0.8) took part in this study. Mean age was 62.3± 2.5 years (M± SD), diabetes duration was 15.6±1.3 years. The influence of such factors as ulcers depth, ancle/brachial index, systolic blood velocity on tibialis arteries (anterior and posterior), TcpO2, severity of peripheral neuropathy was analyzed. Wagner's wound classification was used to asses ulcers depth. Ultrasound doppler was used to measure blood velocity on tibialis arteries and to estimate ankle/brachial index. TcpO2 was used to asses tissue oxygenation. The NDS was used to diagnose and to quantify peripheral neuropathy. All the patients was divided into two groups: the 1st - healed patients (n=13); the 2 nd-failed patients (n=13), among them 3 patients (23%) had toes amputations, 10 patients (77%) had amputations above ankle. Results. Ankle/brachial index (0.6±0.04 vs 0.5±0.06, ns), systolic blood velocity on tibialis anterior arteres (28.3±5.4 vs 30.6±5.8 m/s, ns),systolic blood velocity on tibialis posterior arteres (22.6±6.6 vs 27.6±8.6 sm/s, ns), severity of peripheral neuropathy (NDS 11.6±1.09 vs 11.54±1.12, ns) does not differ between the two groups. Depth of foot ulcers regarding Wagner classification was significantly lower in the healing patients group (2.8 ± 0.2 vs 3.7±0.11, p<0.001). TcpO2 was significantly higher in the 1 st group (33.8±4 vs 19.8±3.06, p<0.01). Sensitivity, specificity, positive prognostic value was calculate for different TcpO2 values . TcpO2 =25 mm.Hg had the highest specificity (92%) and positive prognostic value (93%) for prediction of healing.

Conclusion. TcpO2 = 25 mmHg and depth of ulcers regarding Wagner classification are the main determinants for neiroischemic foot ulcers healing.

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APPLICATION OF CULTURED KERATINOCYTES - THE NEW METHOD IN THE TREATMENT OF SKIN DEFECTS IN THE SYNDROME OF DIABETIC FOOT.

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Aims of the study were : - to cultivate keratinocytes for creating the artificial sheet for covering of the skin defects in the syndrome of diabetic foot
- in first three patients to test the biocompatibility, presence of infection and gain the general feeling about this method (in medical practice)

Material and methods: Cultured epidermal grafts have been used for the treatment of severely burned patients from the beginning of eighties. We have developed the new methodology of cultivation and transfer of keratinocytes on polymer support. Hydrophilic polymer material (polyHEMA) was selected as polymer carrier because of its good biocompatibility. This new strategy is aimed at reducing the time spent in culture (subconfluent grafts) and improving the handling properties. The keratinocytes are applied to the wound bed hyperproliferative, without enzymatic detachment and are able to colonize the wound rapidly. The polymer carrier acts after the application as an optimal cover for the transplanted cells.

Results: Three patients with the deep skin defect on their foot (area 10-250 cm^2) were treated with cultured autologous and/or allogenic keratinocytes. The time of healing was from 2 to 12 month. Preliminary there was found: 1) biocompatibility of these grafts and no signs of rejection; 2) no signs of infection under the grafts during the treatment. Even if it is difficult to develop control system for measurement of effectivity of cultured keratinocytes the general feeling about first three patients was fast and effective healing of the defect.

Conclusion : Our first experience show that the application of keratinocytes attached to the polymer support represent perspective method for the treatment of skin defect in the syndrome of diabetic foot

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ALGOSTERIL® DRESSING VERSUS VASELITULLE® GAUZE FOR THE TREATMENT OF DIABETIC FOOT LESIONS.

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No comparative trials have been conducted in the treatment of diabetic foot lesions. We carried out a controlled randomised clinical trial to evaluate the healing efficacy and safety of Algosteril dressing versus a neutral greasy dressing for the treatment of diabetic foot lesions measuring 1 to 50 cm^2 and requiring debridement. The two groups were comparable at baseline with respect to demographic characteristics and disease (percent granulation tissue, wound surface size, and TcPo2). Thirty-nine patients were included in the Algosteril dressing group and 38 in the Vaselitulle group. Sixty-nine patients continued the study until week 4 (36 in the Algosteril group and 33 in the Vaselitulle group). The primary assessment criterion was the proportion of patients with granulation tissue covering over 75% of the wound and a 40% decrease in wound surface size. Secondary assessment criteria were pain on dressing change and number of dressing changes required. The results were as follows:

Assessment criteria	Algosteril	Vaselitulle	p
Primary:			
- granulation tissue > 75% + wound surface reduction ≥ 40%, % patients	42.8	28.5	0.21
- granulation tissue > 75%, % patients	44.4	36.3	0.04
Secondary:			
- pain on dressing change, scale 0-10 *(0: no pain; 10: very painful)*	0.3 ± 0.1	1.8 ± 0.6	0.047
- number of dressing changes	20 ± 1.1	23 ± 1.2	0.07

There were four adverse event reports in the Algosteril group, as compared with seven in the Vaselitulle group (events were not related with study treatment). Thus, comparatively with Vaselitulle, Algosteril dressing showed a better clinical result and a better tolerance. In conclusion, Algosteril dressing is appropriate for topical treatment of diabetic foot lesions.

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COST-EFFECTIVENESS OF DERMAGRAFT® FOR THE TREATMENT OF DIABETIC FOOT ULCERS IN FRANCE

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Aims : To assess the cost-effectiveness of Dermagraft® (human dermal replacement) compared with current practice.

Material and methods : A Markov model was developed, to simulate the health status of a cohort of 100 patients with a diabetic foot ulcer, during 52 weeks. The health states considered are : healed, same site recurrence, unhealed not infected, cellulitis, osteomyelitis, amputation and death. Each week, the patient may move among states according to a set of transition probabilities derived directly from the US clinical trial. The costs of each health state were estimated by a Delphi panel of diabetologists (including direct costs only) and valuated in a societal perspective.

Results : The total number or ulcers healed is first ulcers healed (69,35 vs 76,38 ; median time to heal is 14-15 weeks compared with 28-19 weeks) plus recurrences which are subsequently healed within the 52-week period (14,29 vs 25,24 ; median time to heal is 3-4 weeks compared with 5-6 weeks). The average expected cost per patient (C/E) with conventional therapy for the 52 weeks period considered is 47,418 French Francs (FF) vs 54,384 FF for Dermagraft® (including 18.200 FF of Dermagraft® treatment and 36.184 FF of conventional treatment). Because Dermagraft® heals more ulcers, the average cost per ulcer healed is lower (53,522 FF vs 56,687 FF). The incremental cost-effectiveness ratio of Dermagraft® ($\Delta C/\Delta E$) equals 387.84 FF.

Conclusion : Dermagraft® is cost-effective because it offers an opportunity to heal ulcers for less than the price that is already paid by the collectivity, using standard practice (56,687 FF).

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ALPHA-LIPOIC ACID IN THE TREATMENT OF DIABETIC NEUROPATHIC FOOT.

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Aim: To determine whether lipoic acid (LA) will reduce oxidative stress in diabetic peripheral nerves, improve neuropathy and examine its effect of diabetic foot therapy. **Materials and Methods**: The effects of the antioxidant alpha-lipoic acid were studied in a 2-months randomized trial in 60 non-insulin-dependent diabetic patients with severe peripheral neuropathy and neuropathic foot who were randomly assigned to treatment with intravenous infusion of alpha-lipoic acid using doses of 600 mg ALA or placebo (PLAC) during 3 weeks and then orally the same doses of 600 mg ALA or placebo during 6 weeks. Measurement of foot ulcers square and volume, neuropathic symptoms (pain, burning, paraesthesiae and numbness) were scored at baseline and then checked every 2 weeks. Increased nerve lipid peroxidation, high levels of total cholesterol, apolipoprotein (apo-B) of human low density lipoprotein (LDL), triglycerides (TG) and depressed enzymes activity were diagnosed in all of them. Lipid metabolism and erythrocytes membrane metabolism changes were observed at baseline state and at the end of 2, 4, 6, 8 weeks period. **Results:** At the end of 8th week period TG and apo-B of LDL levels were decreased more significantly in patients from the 1st group. Also, we observed more significantly improve of peripheral neuropathy tests. The total symptom score (pain, burning, paresthesia, and numbness) in the feet decreased significantly from baseline to 4th week in ALA 600 mg vs. PLAC. Each of the four individual symptom scores was significantly lower in ALA 600 mg than in PLAC after 4 weeks (all $P < 0,001$). Also, at the same time, healing processes of diabetic foot ulcers were significantly better and quicker in ALA 600 mg than in PLAC ($P < 0,05$). **Conclusions:** This study suggests that treatment with alpha-lipoic acid is effective in reducing symptoms of diabetic peripheral neuropathy and significantly improves healing processes of foot ulcers in non-insulin-dependent diabetic patients.

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The role of orthotic therapy in minimising the risk of ulceration in the diabetic foot

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Aims To evaluate the therapeutic effectiveness and cost utility of orthotic therapy in minimising the risk of plantar ulceration.

Materials and Methods 150 diabetic patients with 'at-risk' feet were provided with special footwear and orthotic therapy. All were randomly allocated either orthoses which provided plantar cushioning or increased plantar surface contact area. The costs of the interventions were noted and SF 36 measures were ascertained at 3 time points for all trial patients. These were compared with measures derived from comparison groups not included in the RCT.

Results 97% (115) of the 118 patients completed the study with positive outcomes. The re-ulceration rate was 2.5% and the overall ulceration rate 1.69%. Differences in the effectiveness of the two therapies did not reach statistical significance. In both groups an overall reduction in lesion surface area was achieved. Those allocated cushioning presented with a mean lesion surface area of 7.61cm^2 at entry, and on completion of 2.52 cm^2; those allocated therapy which increased the plantar surface contact area presented with lesion surface area of 8.37cm^2 at entry and 2.97cm^2 on completion. All trial patients demonstrated an improved health status whereas the comparison groups deteriorated ($p<0.05$). The cost of the intervention was £502 per patient over the 18 month intervention and 0.1 additional QALYs generated during the first year. Over the lifetime of the footwear (two years) the cost per QALY ranges from £1.673 to £2.510.

Conclusions This study provided well-documented evidence on the positive effects of orthotic therapy in reducing lesion development in the diabetic foot. It demonstrated reduced superficial lesion formation (hyperkeratosis) and ulceration in a population at high risk at low cost, thus meeting the targets of the St Vincent Declaration. It secured significant improvements in health status.

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INTEREST OF HYPERBARIC OXYGEN THERAPY IN THE TREATMENT OF CHRONIC DIABETIC FOOT ULCER: A RANDOMIZED STUDY

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Hyperbaric oxygen therapy (HBOT) decreases the major amputation in diabetic patients with severe gangrenous foot. Stimulating both angiogenesis and fibroblastic proliferation, HBOT is of interest in the treatment of diabetic foot. **The aim** of the present study is to evaluate the influence of HBOT on the healing delay of chronic diabetic foot ulcer. **Patients and Methods**: this prospective randomized study concerns the type 1 and 2 diabetic patients with chronic foot ulcers (Wagner grade I, II, III) for at least 3 months. Theses ulcers are characterized by the absence of favourable evolution despite a satisfactory metabolic, infectious, podologic and vascular control. Two sessions of 90 min/day (O_2=100%; 2.5 absolute atmosphere) are performed during 2 weeks in the group randomized for HBOT. After one month, the size (area and depth) of ulcer is measured using a standardized photography of the wound associated to magnetic resonance imaging. The favourable evolution is defined as a reduction of at least 30% of the wound size. **Results**: March 1st, 1998, 20 patients are included in this study. 12 diabetics receive HBOT (sex ratio= 7/5, aged= 64.8±12.5 years, diabetes duration=18.5±6.6years). 90% of these patients are type 2 diabetics and exhibite neuropathy and arteriopathy respectively in 100% and 80% of the cases. The control group are composed of 8 diabetic patients (sex ratio=4/4, aged= 55.3±11.7 years) comparable to the HBOT group. The evolution is favourable in 75% of the patients in the HBOT group versus 50% in the control group. Osteitis is present in both HBOT and control group respectively in 40% and 14% of the cases. Neither adverse effect nor complication are observed. Two patients stoppe their treatment respectively after 10 and 15 HBOT sessions. **Conclusion**: HBOT seems to accelarate the healing of chronic diabetic foot ulcer. To confirme HBOT efficiency, multicentric studies with more patients are required.

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Economics of a New Treatment for Diabetic Foot Ulcers: The Cost-Effectiveness of Becaplermin in Sweden

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Introduction: Treating diabetic foot ulcers is costly, especially when non-healing wounds lead to amputation. Becaplermin, a new recombinant human growth factor, has shown an ability to increase the rate of complete ulcer closure in many patients. Becaplermin treatment, hence, has the potential to save costs associated with long healing times and with amputations.

Objective: Compare becaplermin treatment with "usual" ulcer care in Sweden to assess its economic profile. Specifically, we ask whether becaplermin therapy is cost saving relative to usual care. If the answer is no, becaplermin can still be economically worthwhile. We ask secondarily, then, whether becaplermin treatment is a cost-effective use of resources.

Methods: This is a modeling study. We develop a Markov computer simulation model based on epidemiological parameters taken from a U.S. study, cost data taken from several Swedish studies and price lists, and treatment efficacy taken from an RCT. We measure the hypothetical number of days that patients in a diabetic cohort have foot ulcers and the associated treatment costs.

Results: Using conservative assumptions, we find that savings from avoided treatment fully compensate for the price of Becaplermin after one year (see table below). The primary area for savings is the reduced consumption of outpatient services (mostly related to changing dressings). In contrast to many other countries, many of these dressing changes are done by visiting nurses in Sweden. Consequently, one should be cautious in applying this result to countries where these conditions do not apply. This work is supported by Johnson & Johnson.

1-Year Costs of Usual vs. Becaplermin Care for Foot Ulcers in Sweden ($1996)

	Usual Care	Becaplermin	Difference
Outpatient	9,292	7,654	-1,638
Inpatient/Home Help	9,678	9,184	-494
Becaplermin	--	1,912	+1,912
Total	18,970	18,750	-220

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THE EFFICACY OF INJECTING LIQUID SILICONE IN THE DIABETIC FOOT; A TWO YEAR FOLLOW-UP.

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Aims: A 24 month follow-up of diabetic patients who completed the first randomised double-blind placebo controlled trial (of 12 months duration) of the efficacy of injecting liquid silicone in the foot is reported. **Methods:** In 28 neuropathic diabetic patients without peripheral vascular disease, a total of 1.2 ml liquid silicone or an equal volume of saline (placebo) was injected under metatarsal head areas with callus. **Results:** At 12 months the plantar tissue thickness (measured by weightbearing ultrasound) had increased by 1.3 mm in the active treatment group compared to the placebo group (0.2 mm) (silicone vs placebo; $p<0.00001$), plantar pressures (by pedobarography) were reduced (-2.2 vs 1.2 kg/cm^2) (silicone vs placebo; $p<0.05$), and there was a greater proportion of patients with a reduction in callus formation (69% vs 45%) (silicone vs placebo; $p<0.003$). At 24 months sixteen patients were re-assessed: the plantar tissue thickness was still increased in the active treatment group (0.9 mm vs -0.8 mm) (silicone vs placebo; $p<0.003$), and callus had completely disappeared in 9 (out of 23) silicone treated sites. However, there was no difference in plantar pressure between the two groups (-0.38 vs -0.72 kg/cm^2) (ns). **Conclusions:** Thus, the great improvement at 12 months after injection of silicone was reduced at 24 months, suggesting that intermittent booster injections may be required in certain high risk feet. It is possible that further atrophy of the plantar fat pads may explain the results seen at certain sites.

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THE EFFECT OF SULODEXIDE ON MICROCIRCULATION AND NEURAL CONDUCTION IN THE DIABETIC FOOT SYNDROME

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Ulcerations in the course of the diabetic foot syndrome are induced by neuropathic, vascular and mechanical factors. Attempts have been made to use various agents improving microcirculation.

Aim. The purpose of the study was to evaluate the usefulness of Sulodexide in the treatment of diabetic foot based upon:
- its effect on microcirculation
- its effect on neuropathy assessed from the neural conduction velocity.

Material and Methods. The study was conducted in two groups - 12 and 6 patients with type 1 and type 2 diabetes combined with advanced neuropathy and ulcerations. The 12 patients had been given insulin plus Sulodexide for a mean of 8 weeks; the 6 patients received the same treatment but without Sulodexide. In each patient we analyzed hyperemia at rest and reactive hyperemia (using a laser-Doppler flowmeter) at 30 and 60 s after arterial occlusion as well as the neural conduction velocity.

Results. Reduced reactive hyperemia at 30 and 60 s after occlusion was observed in the feet with ulceration as compared to the feet without ulceration. After 60 sec. occlusion higher increase percent of LDF (laser Doppler flow) in group with insulin + sulodexyd in comparison to insulin + placebo was observed (218 +/- 26.4% and 164+/-15.4% respectively, $p = 0.013$). No improvement of the neural conduction was observed in either group throughout the 8-week treatment.

Conclusion. Both improved metabolic normalization in diabetes and Sulodexide have beneficial effects on reactive hyperemia in the diabetic foot. In those receiving Sulodexide the improvement was statistically significant.

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MANAGEMENT OF THE NECROTIZING DIABETIC FOOT INFECTION

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AIM. To find more efficient and cost-effective management of necrotizing diabetic foot (DF) infection METHODS. Three groups of NIDDM patients (age 48-82 years) with grade 2-4 of foot lesion (after Wagner) were studied: 1) In control group (n=57) traditional surgical debridement (SD) was applied; 2) In group 2 (n=40) surgical (35 Watt) CO2-laser "Scalpel -1 or -3" (Russia) was used for SD with following wound irradiation by non-focused laser beam (up to 30 Watt/cm2), and then on days 2 and 3 intravenous laser irradiation of blood using helium-neon laser (0.5 Watt during 30 min); 3) In group 3 (n=30) lasers were used, like in group 2, plus i.m.injection 100-200 mg/d of MEXIDOL were utilized on days 4-8 after SD. Mexidol (Russia) is 3-oxipyridin derivative and is used as antioxidant and antiaggregant. There were 50% neuropathic foot, 50% - neuroischemic in each group. All patients received adequate insulin and antibiotic therapy. The wound healing and infection eradication rates were evaluated. The foot microcirculation was assessed by laser Doppler fluxmetry. The degree of oxidative stress was evaluated by measurement of malonyl-dialdehyde (MDA) and ceruloplasmin (Cer) level and blood activity of xanthine oxidase (XO), GSH-peroxidase(PER) and catalase (Cat). RESULTS. The wound bacterial flora estimated on days 2-3 after SD: 10 - 100 millions of bacteria were found in 1 g of tissue in group 1 and only 100-0 - in groups 2 and 3. Complex index of microcirculation increased 2.8-fold in group 2 and 4-fold in group 3 on days14-21. MDA and XO levels were half as much in group 3 compared to group 1 on days 8-14. PER, Cer, Cat activity increased 1.9, 1.5, 2.5-fold, respectively in group 1-3. The granulation and epithelialisation began on days 3 and 5 in group 3 and on days 7.4 and 9 in group 1. The number of repeated surgical interventions decreased. Complete restoration of foot function was evident in 4.8, 17.8 and 30.0%, respectively in groups 1-3. Above-knee amputations were performed in 13, 5 and only in 3 patients of groups 1-3, respectively. THUS, addition of SD by laser, intravenous laser irradiation of blood and mexidol to management protocol of DF infection significantly accelerate bacterial eradication and wound healing rate, improve microcirculation and antioxidative defence. Such protocol is the cost-effective way of improving of the outcomes of the lesions in DF infection.

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Diabetic Foot – Microbiology and General Features

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Gram-stain for Diabetic Foot Infections: Relevant, Reliable or Relic?

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Aims: The purpose of this manuscript was to identify the prevalence of correlation between Gram's stain and final deep tissue cultures in diabetic foot infections. **Materials and Methods:** We abstracted data from 100 consecutive subjects, 78% male with a mean age of 49.3 ± 11.1 years, admitted to a university teaching institution for operative debridement of an infected diabetic foot. All Gram's stains and cultures were secured intraoperatively and simultaneously from the same anatomic location. Twenty percent of isolates were secured from bone, 54% from soft tissue, and 26% from exudate deep within an abscess. All microbiological assessment was performed within the same laboratory. **Results**: Of 100 consecutive culture results, a total of 66% of Gram stains reported fewer organisms than the final culture and 17% reported more organisms than the final culture. In 48% of cases, the Gram stains did not correlate with the final culture in terms of gross Gram positive/Gram negative characteristics. There was not a significant difference between prevalence of correlation whether the final cultures grew Gram positive, Gram negative, anaerobic, or a combination of these classes of microorganisms. **Conclusion**: We conclude that in a majority of cases, the Gram's stain may provide an incomplete or inaccurate picture of the actual growth of microbes existent in the infected diabetic foot wound. Furthermore, in a stepwise approach to treatment of these infections, which often consists of broad-spectrum empiric followed by culture-directed narrow-spectrum antibiotic therapy, the Gram's stain is of limited value and may not be a cost-effective implement.

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METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS: AN INCREASING PROBLEM IN A DIABETIC FOOT CLINIC

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Aim: To study the prevalence of pathogenic organisms, as well as the prevalence and outcome of methicillin-resistant staphylococcus aureus (MRSA) infected diabetic foot ulcers. **Methods:** A retrospective analysis of wound swabs performed in infected diabetic foot ulcers. Patients were selected from an out-patient diabetic foot clinic. Seventy-five patients (79 ulcers) with positive wound swabs were included in the study. Size of ulcers and time to healing, in particular MRSA infected ulcers, were measured in all patients. **Results**: Gram-positive aerobic bacteria were the commonest micro-organism isolated (56.7%) followed by Gram-negative aerobic bacteria and anaerobes (29.8% and 13.5% respectively). Of the Gram-positive aerobes, Staphylococcus aureus was found most frequently and 40% were MRSA. MRSA was isolated more commonly in patients treated with antibiotics before the swab was taken compared to those who did not receive antibiotics (p = 0.01). Patients whose foot ulcers were infected by MRSA had longer healing time than patients whose ulcers were infected by methicillin-sensitive Staphylococcus aureus [mean (range): 35.4 (19-64) and 17.8 (8-24) weeks respectively, p = 0.03]. **Conclusion:** MRSA infection is common in diabetic foot ulcers and is associated with previous antibiotic treatment and prolonged time to healing. Further studies are urgently required to assess the need for antibiotics in diabetic foot ulcers and also to assess the optimum therapeutic approach to this increasing problem.

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ANTIMICROBIAL THERAPY WITHOUT SUSCEPTIBILITY PATTERNS IN DIABETIC FOOT INFECTIONS ?

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Aims: Soft tissue and bone infections in lower limbs are severe diabetic complications with a high impact on quality of life and the sozioeconomic system. Very often patients with these problems are not treated at all or are treated insufficiently. Recent studies investigated the most common isolates from wounds in diabetic patients and the sufficient antimicrobial therapy. However there are no clear recommendations as to whether bacterial analysis of the wound should always be performed prior to antibiotic therapy. We investigated therefore typical isolates in different wound stages (Classification of Wagner). **Material and Methods:** Retrospectively 111 cultered swabs from 86 patients from 1996 to 1998 were evaluated. **Results:** Altogether 201 organisms could be isolated. The most common isolate was Staph. aureus (n=39 / 19,4%) followed by Coag. Staph. (n=32 / 15,9%), Enterococcus (n=18 / 8,9%), Streptococcus (n=15 / 7,5%), Corynebacteriacae (n=12 / 5,9%), followed by gram-negative bacteria like Pseudomonas aeruginosa (n=12 / 5,9%), E.coli (n=10 / 5%), Klebsiella and Proteus (n=8 / 4%each), Stenotrophomonas maltophiliae (n=3 / 1,5%), other Enterobacteriacae (n= 7 / 3,5%) and anaerobic organisms like Peptostreptococcus (n=7 / 3,5%) and Bacteroides (n=2 / 1%) of patients with lesions. There was a median of 2,45 isolates per wound (range 0-8). In Wagner stage 2 (n=17 patients) were 81% gram-positive isolates, 19% gram-negative, no anaerobic bacteria, in Wagner stage 3 (n=46 patients) 54% gram-positive, 32% gram-negative and 14% anaerobes and in Wagner stage 4 (n=22 patients) 46% gram-positive, 40% gram-negative and 14% anaerobes were isolated. There was no patient with Wagner stage 5 and only one patient with Wagner stage 1. According to the susceptibility patterns most patients (> 85%) received ofloxacin, ciprofloxacin, clindamycin or amoxicillin/clavulanic acid. Only in less than 5% multiresistant bacteria were present. **Conclusion:** We suggest that these data are sufficient for an immediate blind antimicrobial treatment. Especially in Wagner stages 1 - 3 without suspected osteomyelitis or systemic infection expensive and often inadequate performed swabs are waste of time and resources. Local wound care, longterm antibiotics and improved patient care are the most important measures in diabetic foot infections.

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BACTERIA IN SUPERFICIAL DIABETIC FOOT ULCERS

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Aims. Cultures from diabetic foot ulcers usually yield mixed flora containing both colonizing bacteria from the surrounding skin and clinicaly significant pathogens. Results obtained from deep tissue samples are regarded as more reliable. Although systemic and local signs of inflammation are often absent in diabetic foot wounds, antibiotic treatment has been shown to enhance wound healing. In order to compare the results obtained by superficial and deep tissue samples, we studied a group of 11 type 2 diabetics (9 men, 2 women, average age 65.4 years, HbA_1c 8%) with foot ulcers (duration 1 month to 3 years). None of the patients had systemic signs of inflammation and the laboratory results were within normal range (WBC 7.40, ESR 37.6, CRP<15). On the X rays, osteitis was shown in 1 patient, and in 2 the x-ray findings were suspicious. Methods: swabs were taken after rinsing ulcer base and surrounding skin with normal saline. Then the ulcer base was flushed with 50% povidone iodine solution and rinsed with normal saline. Tissue from base of decontaminated ulcer was obtained by curetting. All the samples were cultured in aerobic and anaerobic conditions. Results: The average number of isolated bacteria from ulcer base swab and curettage was 2.45 and 2.72, respectively. The results were identical only in 2 cases, while in 4, anaerobes were isolated by curettage in addition to the aerobes found also by swabbing. Only in 1 case, there were anaerobes also in the sample obtained by swabbing. All the isolated anaerobes were susceptible to metronidazole. The most common aerobic isolates were Staphylococci and Streptococci. Conclusion: our analysis has proved that anaerobes are often present in diabetic foot ulcers but are not readily isolated form superficial samples. Wound curettage is a more reliable method in comparison with swabbing and could therefore be recommended also for everyday clinical practice.

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DIABETIC NEUROPATHY AND OSTEOPOROSIS IN THE DIABETIC FOOT

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Aims: To correlate the bone mass density (BMD) in the calcaneum in patients with and without neuropathic diabetic foot (NDF) **Material and Methods**: 31 DM patients were evaluated and divided into two homogeneous groups: one with NDF (group 1), the other without NDF (group 2). Group 1: 15 patients: 12 type 2 DM and 3 type 1 DM, 9 male and 6 female mean age 60.6±8.5 yrs. Group 2 :16 patients, 13 type 2 DM and 3 type 1 DM, 14 male and 2 female, mean age 53.2±11.7 yrs. The average of HbA1c, BMI, duration of diabetes and the prevalence of the others complications (retinopathy and nephropathy) were also evaluated. Bone mass density (BMD) was measured at L2 – L4 vertebral and proximal femur by Dual Energy X-Ray Absorptiometry (DEXA) and on the left calcaneum by Ultrasonography. **Results**: in group 1 the averages were: HbA1c (9.1±1.7), BMI (25.3±3.8) and duration of DM (13.4±8 yrs). 11 (73.3 %) patients had retinopathy and 3 (20%) had nephropathy. BMD at vertebral. and femur were normal in 4 patients (26.7%) moderate degree of osteoporosis in 8 patients (53.3%) and osteoporosis in 3 (20%). BMD on the calcaneum was lower in 8 patients (53.3%) while 7 patients showed normal values (46.6%). In group 2 the averages were: HbA1c (9.2±2), BMI (26.3±4.5) and duration of DM (16.8±12 yrs). 8 patients had retinopathy (50%) and 4 had nephropathy (25%). BMD at vertebral and femur were normal in 12 patients (75%) and moderate degree of osteoporosis in 4 (25%). BMD on the calcaneum was moderately low in 3 patients (18.7%) and 13 patients had normal values (81.3%). The difference in BMD on calcaneum was statistically significant between groups 1 and 2 (53.3% VS 18.7% p=0.022), being the higher degree of osteoporosis in the group with NDF. **Conclusions**: Thus we concluded that peripheral neuropathy could be a risk factor for osteoporosis in the diabetic foot.

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Mortality in Diabetic Foot Patients: Major Difference between Ischaemic and Neuropathic Patients

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There is an increased mortality associated with diabetic foot ulcers but the cause is unknown. This study shows that there is a major difference in the survival of patients with ischaemic foot ulceration compared with neuropathic foot ulceration. We reviewed the 5-year survival of 191 patients presenting to the Diabetic Foot Clinic in November and December 1993. Ninety four patients presented with ischaemic foot ulceration (Ankle-brachial pressure index <1.0) and 97 with neuropathic foot ulceration. The mean age was 69.6 ± 9.3 years Mean ± SD in the ischaemic patients and 61.5 ± 12.5 years in the neuropathic patients ($p<0.01$). The 5-year mortality was considerably increased in the ischaemic patients, 44/97 (45%) compared with 20/94 (21%) in the neuropathic patients ($p<0.001$). The mean age of death was 74.6 ± 10.7 years in the ischaemic patients compared with 67.4 ± 13.3 years in the neuropathic patients ($p<0.05$).

In a prospective study of 52 patients with ischaemic ulcers and 57 with neuropathic ulcers serum total cholesterol was 5.24±1.31 v 4.99±1.12mmol/L respectively ns, LDL cholesterol was 3.03±0.82 v 2.68±0.63 mmol/L respectively $p<0.05$, and fibrinogen 4.67±1.13 v 4.41±1.16 g/L respectively ns.

In conclusion, survival of diabetic foot patients is strongly influenced by clinical presentation. Ischaemic foot ulcer patients have twice the mortality rate of the neuropathic foot patients with significantly raised LDL cholesterol.

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OUTCOME FOLLOWING MAJOR LOWER EXTREMITY AMPUTATION FOR DIABETIC AND NON-DIABETIC PERSONS.

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Aim: To establish the outcome for diabetic and non-diabetic persons for mobility, morbidity & mortality following major lower extremity amputation (LEA). **Methods:** Retrospective analysis of six years data from rehabilitation centre following major LEA resulting from peripheral vascular disease and/or infection. A consultant supervised rehabilitation, following the first or only operation. The outcome was graded objectively, from normal to unsuitable for prosthesis. **Results:** 94 diabetic patients and 98 non-diabetic arteriopaths had major lower limb amputations, excluding toe and foot operations. Comparing diabetic versus non-diabetic: males 78% v. 65%, median (range) age at first amputation 67 (38-86) v. 73 (32-94) years: second amputation needed in 18 v. 7 persons, and median (range) interval between amputations 15 (3-57) v. 5 (0-55) months. Comparing diabetic and non-diabetics respectively for amputation sites were: below knee (BKA) 91 v. 54, above knee (AKA) 19 v. 56, through knee 1 v. 2 and through hip 1 v. 0 (odds ratio 5.35 (95%CI 2.79-10.34), $p<0.0001$). Mobility outcome for diabetic v. non-diabetic respectively: near normal in 2 v. 0, independent indoor and outdoor walking 3 v. 1, indoor and outdoor walking with aids 30 v. 18, independent indoor walking only in 22 v. 21, wearing prosthesis only for transfer or to assist nursing in 22 v. 9, cosmetic use only in 5 and 19, v. no prosthesis due to severe illness or death in 10 v. 30 respectively (chi^2 for trend 18.4, $p<0.001$). Deaths during six years were 45 (48%) in diabetic and 51 (47%) in non-diabetic patients. **Conclusions:** The high mortality in both groups probably reflects the consequences of severe generalised vascular disease. Persons with diabetes were more likely than other arteriopaths to have BKA and to require further amputations.

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DIABETIC FEET ARE BROADER THAN NORMAL FOOTWEAR

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Aim: to measure the dimensions (lenght, width) of the feet of 568 diabetic patients(229 women mean (SD) age 64(19)years)with neuropathy.Feet with Charcot-fractures and other deformities were excluded. **Methods:**a rectangular automatic measuring frame(WMS, PFI Pirmasens, Germany) was used, indicating foot length and width in mm, and the length-derived shoe size in French numbers 35 to 46.Foot dimensions were compared to shoe dimensions as found in industry standard tables (Fagus Co. Alfeld,Germany),ranking shoe-widths from class F (small), to classes G, H, I, K, L, M (extremely large, not available for normal footwear)by shoe-number.Class G is the commonest width of normal footwear of any number. **Results:** foot lengths varied between 228 mm (= shoe number 35) and 297 mm (= shoe number 46). Only 10(13)% of male and 11(9)% of female feet fitted width G. In men /women,the mean % prevalence of foot widths over all shoe numbers was F:11(7)/6(6);G: 10 (13)/11(9); H:15(7)/13(5); I:14(7)/20(7); K: 14(8)/13(4); L: 12(6)/10(5); M: 12(7)/9(7); >M: 13(7)/9(4).**Conclusion:** most diabetic feet were broader than shoe width G,and even broader than width H (extra-large).Here is one of the causes, why (normal) footwear contributes so much to injuries of the diabetic foot:it simply is too narrow.

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Laterality of Diabetic Foot Ulceration. Fact or Fiction?

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Aims Diabetic foot ulcers are common and carry enormous social, psychological and financial costs. Recently it has been suggested that within the diabetic population amputations occur 4 times more commonly in the right lower limb compared to the left side. Such laterality could be a consequence of abnormal pressure loading on that side, onto an 'at-risk' foot. If this true, ulceration would be expected to be more common on the right side.

Methods 51 consecutive diabetic patients with active lower limb ulceration were examined according a standard protocol including Doppler measurement of ABPI' s and assessment of peripheral neuropathy using Semmes-Weinstein monofilaments.

Results (mean±SD)

Age	69±13 years		
Gender	19 men		
Type of diabetes	8 type 1		
Duration of diabetes	17±13 years		
Causative factor	Neuropathy	25/51	(49%)
	Vasculopathy	10/51	(20%)
	Mixed	16/51	(31%)
Side	**Left**	**20/51**	**(39%)**
	Right	**25/51**	**(49%)**
	Both	**6/51**	**(12%)**

Conclusion Laterality is not a factor in the aetiology of diabetic foot ulceration.

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HAND SEPSIS IN AN ADULT, TANZANIAN, DIABETIC POPULATION.

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Background: Hand infections in patients with diabetes (DM) in sub-Saharan Africa have not been adequately documented. However, published data suggest that morbidity and mortality in diabetic patients that are hospitalized with hand sepsis could be very high.

Aims: To (1) characterize the epidemiology and clinical characteristics of hand sepsis in an adult diabetic population; (2) document risk factors associated with hand infections.

Methods: Consecutive patients attending a diabetic clinic during 6/98-2/99 (study period) were evaluated for hand infections. A case-patient was defined as any diabetic patient with signs of cellulitis, ulceration, or gangrene in the hand. Control-patients were randomly chosen diabetic patients with no history of hand symptoms. Case- and control-patients were matched by age and sex. Details of history included information on demographics, type and duration of DM, family history of DM, precipitating causes, such as trauma or insect stings, underlying risk factors, such as alcohol and tobacco use, and occupation. Each patient underwent a physical examination that included evaluation for the presence of peripheral neuropathy (PN), microvascular and peripheral vascular disease, and evaluation of hands for sepsis and ulceration. Data were entered in a standardized questionnaire.

Results: During the study period, 19 patients met the case-definition; 57 control-patients were identified. The median age of case-patients was 51 (range 28-76) years; 56% were female. Eight (42%) case-patients were housewives. Precipitating causes included boils (n=5), innocuous looking papules (n=2), burns (n=2), or trauma (n=2). Case- and control-patients were similar for region of residence, presence of micro and macrovascular disease, and alcohol or tobacco use. Compared with control-patients, however, case-patients had a significantly lower median body mass index (23 vs. 28, $p < 0.01$), a higher median duration of DM (72 vs. 21 months, $p < 0.05$), and were more likely to have PN ($p < 0.05$), to be on insulin ($p < 0.01$), or to have a family history of DM ($p < 0.05$). Seven (37%) case-patients underwent incision and drainage, 4 (21%) required amputation, and 4 (21%) died from overwhelming sepsis.

Conclusions: Hand infections in diabetic patients are always serious and should be treated aggressively. Improperly treated hand infections can lead to disability, limb amputation, or death. Diabetic patients should be educated on proper hand care and the importance of consulting a doctor during the early stages of hand-associated problems.

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Retinopathy – Screening and Prevalence

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PARTNERSHIPS IN CARE - DIABETIC RETINOPATHY SCREENING FOR THE NEW MILLENIUM

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Diabetic retinopathy is the commonest cause of visual handicap in the UK. The **aims** of the study were to assess the impact of developing a retinopathy screening programme for patients whose care was supervised solely by their GP and who were not routinely receiving annual dilated fundoscopy. Patients without significant retinopathy would then be referred to an Accredited Optometrist for prompted annual review. **Patients and Methods:** Patients were referred from local GP's for screening. **Results:** To date 1166 patients have been screened. The median (range) age of patients was 68.4 (31-90) years. 65% had no retinopathy. Abnormalities included: mild/moderate retinopathy 143 (12%), significant retinopathy 96 (8%), cataracts 105 (9%), glaucoma 34 (3%), other conditions (mainly ARMD) 35(3%). 187 (16%) patients needed referral to a specialist ophthalmologist for: significant retinopathy (96), glaucoma (34), cataracts (57). Patients with significant retinopathy comprised 38% white, 11% black Caribbean, 29% Greek and Turkish Cypriot, 21% Asian subcontinent and 1% other. **Conclusions:** These results indicate there is a high level of undiagnosed but treatable retinopathy in patients supervised solely within General Practice. More than 50% of patients with retinopathy came from ethnic minority groups. **Recommendations:** Pre-screening patients prior to referring them to prompted Accredited Optometrists is recommended not only to target patients from ethnic minority groups but also to enable them to be 'fast tracked' to a specialist Ophthalmologist.

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TELEMEDICINE FOR DIABETIC RETINOPATHY IN JUVENILE DIABETES USING A STANDARD 9-FIELD FUNDUS PHOTOGRAPHY

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Aims: To evaluate a new standard 9-field non-mydriatic fundus photographing system for diabetic retinopathy in children by telemedicine. **Methods:** 9 internal fixation targets were newly installed to the non-mydriatic fundus camera (TRC-NW5S, TOPCON, Japan). Nine 45° multi-field fundus photos were taken in 81 children (14.5±3.1yrs, HbA1C 7.9± 1.3%) during the summer camp at the rural area of Japan and stored as digital images. Images were edited in 3x3 form on a personal computer and sent to ophthalmologists resided about 200km away through the internet for the diagnosis on the monitor(M). The central field was printed out as conventional fundus photo (C) and compared (quality(Q): evaluated in 5 grades;5.full). **Results:** 9-field photographs could be taken in 76(94%) IDDM children on both eyes. Of 5 patients who could not follow 9 targets, 4 had no patience and 1 was mentally retarded. On average, 176±73sec. was needed for taking a series of 9 fundus photos and 74±7sec. for sending one 3x3 image (average size: 243±22kb) through the internet. The average pupil size was 5.8±0.7 mm. Q was significantly better on M(4.9±0.3) than on C(4.7±0.6)($p<0.005$) in one doctor. In 141/152(93%) eyes, agreement among the doctors was made about the absence of retinopathy. 11 eyes were diagnosed as simple retinopathy both on C and M. The percentages of agreement in the diagnosis by 2 out of 3 ophthalmologists tended to be higher on M (7/11(64%) eyes) than on C(3/11(27%))($p=0.087$). At the peripheral fundus area on the monitor, two retinal holes were diagnosed and quick referral to the ophthalmologist was made. **Conclusions:** A new system, the standard 9-field non-mydriatic fundus photography and transmission of retinal images through the internet, was applicable to screening of retinopathy in juvenile diabetes, especially at the rural areas.

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SCREENING FOR PATIENTS WITH RETINOPATHY AND POOR DIABETES CONTROL - JELAS I AND II, AN INTERVENTION-TRIAL IN 380 PATIENTS

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In many European countries diabetic retinopathy is a major cause of blindness. Most retinopathic blindness is preventable by early laser therapy and improvement of diabetes control. During the first 3 months of 1997 we assessed in a cross-sectional study (JELAS I) all diabetic patients (n=216) attending the ophthalmologic out-patient department for laser therapy of our clinic. HbA1c-values (HPLC, Diamat®, normal range 4.4-5.9%) above 7.5% were found in 88% (21/24) of the patients with type 1 and in 82% (157/192) of the patients with type 2 diabetes. In a feasibility-study a standardized letter was sent to the primary care physicians of all the patients with HbA1c-values higher than 10% (type 1/2: n=11/52). These letters included the informations about patients' actual HbA1c-value, the importance of good quality of diabetes care to prevent the progression of retinopathy and options to improve quality of diabetes control. One year after this intervention all the patients were re-examined and HbA1c was measured: An improvement of diabetes control was found in 35% of the patients. Following the faesibility-study the JELAS II-trial was started in 1998: Again, all the patients (n=164) successively attending the ophthalmologic out-patient department for laser therapy were investigated. HbA1c-values higher than 9% were found in 8/20 of the patients (40%) with type 1 and in 61/144 of the patients (54%) with type 2 diabetes. Now, the standardized letter was not only sent to the primary care physicians but also to the patients: This increased the intervention rate to 55% ($p<0.05$) up to the time of re-examination of all the patients one year later. **Conclusions:** The ophthalmologic department is a possible link to the internal medicine: Here, with a small screening programme patients with poorly controlled diabetes must be identified. For many patients with severe retinopathy, this is the last chance to avoid a further loss of visual acuity and blindness. Efforts to improve diabetes control must be started in cooperation with the patients, primary care physicians and diabetologists.

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ORAL FLUORESCEIN ANGIOGRAPHY AS AN ADDITIONAL TOOL IN DIABETIC RETINOPATHY SCREENING.

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Aims: To assess the feasibility of using oral fluorescein angiography to detect macular oedema as part of the diabetic retinopathy screening process in the hope that by doing this we can greatly reduce the number of referrals to an ophthalmologist. **Materials and Methods:** The patients were a consecutive series of patients who should be referred to an ophthalmologist according to the standard European referral criteria, in particular haemorrhages or hard exudates within one disc diameter of the macula during screening using retinal photography combined with ophthalmoscopy. Oral fluorescein sodium at 25mg/kg in capsule form was administered followed by posterior pole photographs using a Topcon Image Net taken at the start and then at 15 minute intervals for one hour. Presence or absence of oedema at or near the macula was assessed on the fluorescein image. Side effects during or after dye administration were noted and quality of image graded according to degree of fluorescence. All patients also underwent slit lamp biomicroscopy by an experienced ophthalmologist (EEK) who was blind to the results of the oral fluorescein assessment. The retinopathy was graded including presence or absence of clinically significant macular oedema (CSMO). **Results:** 22 patients have had the investigation to date. 4/22 (18%) patients had dye leakage suggesting macula oedema on oral fluorescein angiography and of these 2 had CSMO on slit lamp assessment and received laser treatment. None of the other patients had CSMO. 8 were kept under ophthalmological follow up for maculopathy without oedema and 12 were discharged. 1 patient experienced rash and urticaria which subsided on administration of an oral antihistamine. Only 1 case had insufficient fluorescence and therefore an inconclusive angiography test. **Conclusion:** These data raise the possibility that oral fluorescein angiography may be a safe and reliable method of assessing presence of macular oedema which could be used in the screening process to greatly reduce the number of unnecessary referrals to ophthalmologists.

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IF THERE IS NO DIABETIC RETINOPATHY PRESENT, COULD THE SCREENING INTERVAL BE INCREASED TO TWO YEARS?

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Aims: One year is traditionally recommended as the diabetic retinopathy (DR) screening interval, although there is little data to support this. As there are resource implications we aimed to investigate the need for this time interval. **Methods:** Information from the diabetic database from Jan.1993 to Nov.1998 was analysed. The data were collected using ophthalmoscopy combined with Polaroid photography (dual modality eye screening). A modification of standard European criteria for referral to an ophthalmologist were used. **Results:** Full eye data were available on 6676 visit sessions. 3959 (59.3%) records were documented as having no DR and 764 (11.4%) were referred to an ophthalmologist. A total of 22 patients required referral for DR, after documented no DR on the previous eye screening. Of these 22 patients, 4 (17.4%) had a screening interval of < 12 months (range 6-12), 5 (20.8%) between 12 -24 months (range 14-18) and 13 (54%) > 24 months (range 24-53). Of the 22 referred, 1 received laser treatment for proliferative DR (PDR), 6 had mild to moderate non proliferative DR(NPDR), 4 had background DR(BDR), 5 did not attend(DNA), 3 are still to be seen and in 3 cases no referral letter was received. Reviewing the 4 patients with screening interval of < 12 months, 1 developed PDR at 6 months and received laser treatment, 2 had NPDR requiring no treatment and 1 mild NPDR DNA. Of the 5 screened between 12-24 months, 2 had mild NPDR, 2 BDR and 1 with mild NPDR at referral DNA. **Conclusion:** The incidence of sight threatening DR within 24 months after documented no DR on dual modality screening was 1 in every 3959 cases examined. Thus if this sensitive screening method shows no DR, a screening interval of 24 months is acceptable in the vast majority of cases.

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Successful implementation of screening for diabetic eye disease – results of the Volkswagen Diabetes Project

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1997 Volkswagen health insurance introduced as a model project an annual health check for people with diabetes aiming at early detection of diabetic complications. It includes screening for diabetic eye disease by ophthalmologists (additional salary for the documentation = 10 Euros). The patients receive a copy of the results, another copy is forwarded for the evaluation, documentation is the prerequisite for remuneration. During one year 1.563 patients were screened (2.57 % of 60.800 persons insured by Volkswagen in the city of Wolfsburg).

Results: Age 65.7 ± 8.7 years, years since diagnosis of diabetes 9.9 ± 7.8, rubeosis iridis 2 eyes, caratact with impairment of visus 24.4 % of eyes, pseudophakia 7.8 %. No retinopathy was diagnosed in 80.9 % of eyes, mild forms of retinopathy in 14.1 %, severe retinopathy 3.3 %, proliferative retinopathy 1.3 %. Macular edema was diagnosed in 41 eyes, vitreous hemorrage in 11, vitreous traction in 7 eyes. After < 10 years following diagnosis of diabetes in 90.3 % of eyes no retinopathy was diagnosed, after 10-20 years in 68.5 %, after > 20 years in 53 %. In only 3.6 % of eyes 20 years after diagnosis of diabetes proliferative retinopathy was diagnosed. Laser-coagulation was recommended in 118 eyes, fluorescein angiography in 17, vitrectomy in 2 eyes.

Summary: Screening for diabetic eye disease including precise documentation was introduced successfully into routine ambulatory care. In both types of diabetes we observed a substantially lower prevalence of severe forms of retinopathy compared to the literature.

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PREVALENCE OF DIABETIC RETINOPATHY IN THE HOORN STUDY

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Aims: To compare the prevalence of diabetic retinopathy in the Netherlands among different categories of glucose tolerance according to World Health Organisation (WHO) and American Diabetes Association (ADA) criteria.

Materials and Methods: The Hoorn study is a population based cohort study of glucose tolerance, which started in 1989 among 2484 inhabitants of Hoorn, aged 50-74. A subsample of these participants (n=625) underwent more extensive baseline measurements during 1989-92, including fundusscopy and 2-field fundusphotography. Prevalence of diabetic retinopathy was determined according to different categories of glucose tolerance status defined by the WHO (1985) and the ADA (1997) criteria.

Results: Known type 2 diabetics (n=77), using insulin or oral medication for diabetes showed a significantly higher prevalence of diabetic retinopathy (33.8%; 95%CI: 28.4 - 39.2) than the other classes in the table. Among newly diagnosed diabetics according to the ADA criteria there was a higher prevalence of diabetic retinopathy.

Table: percentage diabetic retinopathy (of total number) among categories of glucose metabolism; according to WHO and ADA.

class	normal tolerance	impaired tolerance	new diabetes
WHO criteria	< 7.8 fasting and < 7.8 OGTT	< 7.8 fasting and 7.8-11.1 OGTT	≥ 7.8 fasting or ≥ 11.1 OGTT
diab. retinopathy	9.8% (285)	14.3% (168)	11.6% (95)
ADA criteria	< 6.1 fasting	6.1-7.0 fasting	≥ 7.0 fasting
diab. retinopathy	9.7% (371)	13.4% (97)	17.5% (80)
combined criteria	< 6.1 fasting and < 7.8 OGTT	6.1-7.0 fasting and 7.8-11.1 OGTT	≥ 7.0 fasting or ≥ 11.1 OGTT
diab. retinopathy	9.8% (256)	11.9% (177)	14.8% (115)

Conclusions: By lowering the fasting cut-off point for the diagnosis of diabetes, a larger proportion of subjects with diabetic retinopathy is detected.

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INCIDENCE OF RETINOPATHY AND RISK FACTORS IN TYPE 1 DIABETES

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Aims: Retinopathy occurs in the majority of patients with type 1 diabetes, but the role of several key risk factors is still poorly understood. **Materials and methods:** We calculated the incidence of retinopathy and examined risk factors in a cohort of 1335 people with type 1 diabetes, defined as an age at diagnosis <36 years with a need for continuous insulin within a year of diagnosis, aged between 15-60 years in Europe and with no retinopathy at baseline. Retinal photographs (2 fields per eye) were taken according to the standardised EURODIAB protocol, and were graded to a 6 level scale at both baseline and follow-up (7.3 years later). **Results:** Follow-up photographs were available from 53% (712/1335) of the original cohort. Baseline distribution of retinopathy and risk factors did not differ significantly between those in whom follow-up data were and were not available. Incidence of retinopathy was 56% (95% CI 52, 60%). Incidence peaked at 15-20 years duration, and declined thereafter. Risk factors for incidence included diabetes duration (11 vs 9 years, p=0.001). The remaining risk factors were adjusted for diabetes duration, these included: HbA_{1c} (7.0% vs 5.6%, p=0.0001), albumin excretion rate (12.3 vs 10.0 µg/min, p=0.007), total cholesterol (5.1 vs 5.0 mmol/l, p=0.05), fasting triglyceride (0.92 vs 0.79 mmol/l, p=0.0004), HDL cholesterol (1.54 vs 1.48 mmol/l, p=0.06) and waist hip ratio (0.87 vs 0.83, p=0.0002). There was no difference in age or blood pressure at baseline, and although the proportion of current smokers in incident cases was higher (33% vs 26%) this was not statistically significant (p=0.07). Incidence of retinopathy was positively associated with HbA_{1c}; those with an HbA_{1c} ≥5.0 and <5.5% had a risk 1.7 (95% CI 0.99,2.97, p=0.06) times greater than those with an HbA1c <5.0%. **Conclusions:** There is no threshold of HbA_{1c} at which retinopathy risk is negligible, and apart from glycaemic control, lipids may also be of importance in the aetiology of diabetic retinopathy.

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NO REDUCTION OF DIABETES-RELATED BLINDNESS IN THE PROVINCE OF TURIN. DID SAINT-VINCENT NOT WORK?

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Aims: a permanent observatory was set up to monitor certified blindness in the province of Turin, North-West Italy. All case notes compiled in 1954-1997 were reviewed to establish the relative weight of diabetic retinopathy (DR) as a cause of legal blindess over time. **Methods**: Sex, age at application for legal benefits, primary and secondary causes of bilateral and monolateral sight loss were collected on an Access for Windows® database. Data were grouped with a view to milestones in DR history: 1954-67 (pre-laser), 1968 (Airlie House meeting)-77, 1978 (DR Study)-82, 1983-87, 1988 (reports from Early Treatment of DR Study)-1992 [Saint-Vincent Declaration (SVD) screening protocols], 1993-97 (SVD implementation in the area). **Results**: 7550 records were available of individuals who had been certified visually impaired (1/20 vision or less in the best eye). DR was the primary cause of visual loss in 793 people (529 women) and a secondary cause in another 43. Causes of bilateral visual loss are shown as percentages of overall blind people:

Years	54-97	54-67	68-77	78-82	83-87	88-92	93-97
No. Blind	7550	168	1366	693	1329	1737	2253
Age	66±21	44±12	59±18	58±24	67±20	67±24	72±19
Cataract	20.0%	13.1%	22.6%	17.0%	22.2%	22.0%	17.1%
DR	10.5%	0%	7.5%	9.7%	10.7%	11.1%	12.8%
Myopia	7.9%	3.0%	6.7%	6.1%	6.8%	9.9%	8.7%
SMD	6.6%	0%	1.0%	1.0%	3.2%	7.2%	13.6%
OA	6.2%	13.1%	8.0%	7.9%	7.3%	5.0%	4.1%
RP	5.3%	13.7%	4.7%	5.5%	6.7%	5.0%	4.5%

SMD=Senile Macular Degeneration; OA=Optic Atrophy; RP=Retinitis Pigmentosa.

Of the above people, 3399 were totally blind (≤hand movement), 340 (10.0%) from DR and similar distributions for other causes. **Conclusions**: despite improved procedures and instruments for early diagnosis and treatment, blindness from DR is rising in Turin. Ageing and reduced blindness from cataract may play a role but inadequate referral chains for photocoagulation are perhaps to blame. More efficient ways to screen for and treat DR must be implemented to attain the SVD goals.

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THE PREVALENCE OF DIABETIC RETINOPATHY IN NEWLY DIAGNOSED TYPE 2 DIABETICS

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Aim: To determine the prevalence of diabetic retinopathy in Type 2 diabetics with duration of diabetes 0 to 2 years

Materials and Methods: We studied the records of newly diagnosed 1289 Type 2 diabetics (aged 33 to 74 years). Patients with a history of glaucoma or uveitis or of an intraocular surgery were not included into this study. All patients underwent fundus examination through dilated pupil using binocular indirect ophthalmoscope with 20 D lens and slitlamp biomicroscope with 78 D lens by the same ophthalmologist. The diagnosis of diabetic maculopathy was confirmed by fundus fluorescein angiography.

Results: The prevalence of diabetic retinopathy was 15.1% Nonproliferative diabetic retinopathy was diagnosed in 13.0% (1.4% with macular involvement), preproliferative diabetic retinopathy in 1.6%, proliferative diabetic retinopaty in 0.5% of patients.

Conclusion: Sight threatening retinopathy may be present in newly diagnosed Type 2 diabetics because of its insidious onset.Patients with Type 2 diabetes should be referred routinely for ophthalmological evaluation at the time of diagnosis.

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THE STUDY OF VISUAL SYSTEM FUNCTIONALITY IN TYPE 1 DIABETIC PATIENTS

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Introduction: the visual system functionality was examined through psychophysical and objective methods in type I diabetic patients, without retinal and general complications due to diabetic pathology. The aim of our study was to search the presence of the functional alterations in the visual apparatus singling out, as much as possible, the first area interested. *Materials and methods*: 60 type I diabetic patients were studied (average age 16.7+/-5.4) through following inclusion's criteria: no clinical evidence of diabetic complications and the absence of the other systemic pathologies; visual acuity at least 10/10; the absence of the anterior segment alterations; the absence of dioptrics means opacity; the absence of *fundus oculi* alterations examined with biomicroscopy of the posterior pole and the indirect dynamic ophtalmoscopy of the periphery; the absence of amblyopia. The selected patients underwent: 1)the study of the sensibility curve in contrast; 2)the study of the Visual Evoked Potentials (VEP) from the pattern televisiv stimulus with three spatial frequencies of the stimulus (0.49;1.45;4.37 C/°); 3)the study of the standard Electroretinogram (ISCEV); 4)the registration of the Scotopic Threshold Response (STR). The results were compared with a normal homogeneous group for the age and then underwent the statistical quantitative analysis through Student' s t-test. *Results*: in all the examined patients it were seen the following alterations statistically significant: the reduction of the contrast sensibility, homogeneous in all the spatial frequencies used; slight alterations of VEP, represented from the width's reduction and the latent increase of all the spatial frequencies of the stimulation; the remarkable alteration of STR, represented from the width's reduction of the wave and the increase of the culmination's time. It were seen some non significant alterations in the standard electroretinographic response. *Conclusions:* the results has clearly showed the presence of a initial damage widespread through the visual apparatus. The area the most damaged from the systemic pathology seems to be the retina, mostly into the internal layers of retina; just from this area originates the STR, produced mostly from the amacrine cells that, in according to some theories, may be responsable of the control of the intraretinic microcirculation. An alteration of the STR show a malfunction of the amacrine cells, that didn't effect a good control to the intraretinic blood circulation. This alteration may cause the trophic alterations of the retinopathy.

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ADRENOMEDULLIN (AM) IN DIABETICS : RELATIONSHIP OF AM, ANP OR BNP TO DIABETIC RETINOPATHY

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Aim : Adrenomedullin (AM) is produced by vascular endothelial cells, and shows vasodilative actions and inhibitory effects on proliferation of vascular smooth muscle cells. Furthermore, factors with similar actions include atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP). We examined the usefulness of measuring AM, ANP and BNP during severity of diabetic retinopathy. **Materials and Methods** : This study included 42 diabetics. With respect to diabetic retinopathy, the subjects were divided into 3 groups, the non-diabetic retinopathy (NDR) group, the non-proliferative diabetic retinopathy (NPDR) group and the preproliferative diabetic retinopathy (PPDR)+proliferative diabetic retinopathy (PDR) group. Plasma AM, ANP and BNP were measured by immunoradiometric assay (IRMA). **Results** : There was a positive correlation between plasma ANP levels and plasma BNP levels ($r=0.775$ $p<0.0001$). However, there were no relationships between ANP, BNP and AM levels. Furthermore, the plasma AM levels in the PPDR+PDR group (14.85 ± 7.39 fmol/ml) was significantly higher than those in the NDR group (10.14 ± 3.27 fmol/ml) or the NPDR group (8.34 ± 1.33 fmol/ml) ($p<0.01$). Plasma ANP levels in the NPDR group (38.20 ± 43.70 pg/ml) and the PPDR+PDR group (40.40 ± 28.31 pg/ml) were significantly higher than that in the NDRgroup (13.91 ± 10.57 pg/ml) ($p<0.05$). Plasma BNP levels in the NPDR group (101.60 ± 201.48 pg/ml) and the PPDR+PDR group (85.29 ± 161.94 pg/ml) were slightly higher than that in the NDRgroup (15.37 ± 15.45 pg/ml). **Conclusions** : These results suggested that increased plasma AM levels in the PPDR+PDR group may have been associated with the vasodilative actions of AM as in vivo response to decreased retinal blood flow. The mechanism involved in secretion of AM may differ from that involved in ANP or BNP secretion.

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LACK OF RELATIONSHIP BETWEEN INTRAVITREOUS IGF-1 AND VEGF IN PROLIFERATIVE DIABETIC RETINOPATHY.

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Elevated vitreous levels of insulin-like growth factor 1 (IGF-1) as well as vascular endothelial growth factor (VEGF) have been reported in patients with proliferative diabetic retinopathy (PDR). The source of high vitreous levels of IGF-1 and VEGF is presumably ischaemic retina, but increased levels derived from serum diffusion due to the disruption of the blood-retinal barrier can not be excluded. **Aims:** To determine whether a relationship between IGF-1 and VEGF exists in vitreous fluid from patients with PDR.**Materials and methods:** Vitreous samples were obtained from 24 diabetic patients with PDR (group A) and 16 nondiabetic patients (group B) in whom a vitrectomy was performed. Both groups were equipared by age, serum IGF-1 and serum VEGF concentrations. IGF-1 was determined by RIA and VEGF was assessed by ELISA. Statistical analysis: Mann-Whitney U test and a Spearman's rank correlation test. **Results:** Vitreal levels of both IGF-1 and VEGF were elevated in group A in comparison with group B (median and range), IGF-1:[1.55 ng/ml (0.3-8.7) *vs.* 0.3 ng/ml (0-1.4); $p<0.0001$] and VEGF: [1.99 ng/ml (0.3-6.6) *vs.*0.009 ng/ml (0.009-0.038); $p<0.0001$]. These results remained at significant level after adjusting by vitreal protein concentration. Finally, a relationship between IGF-1 and VEGF was not observed.**Conclusions:** Our results suggest that intraocular synthesis contributes to elevated vitreous levels of IGF-1 and VEGF but there is a lack of relationship between vitreous concentrations of these two angiogenic factors.

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DOSE-DEPENDENT EFFECTS OF TAURINE IN THE PREVENTION OF NA+-K+-ATPASE IMPAIRMENT AND LIPID PEROXIDATION IN THE RETINA OF STREPTOZOTOCIN-DIABETIC RATS.

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Aims: To study the susceptibility of diabetic retinas to neural malfunction and free radical-induced damage in the form of lipid peroxidation, we evaluated the activity of membrane-bound enzymes such as Na+-K+-ATPase and the metabolism of lipid hydroperoxides (LPs) and conjugated dienes (CDs) in retinas from nondiabetic and streptozotocin (STZ)-induced diabetic rats given a standard diet or a 2% and 5% (w/w) taurine-supplemented diet. **Materials and Methods:** Rats were sacrificed and their retinas removed at 2, 4, 8, and 16 weeks. With spectrophotometric methods, we measured the ouabain-sensitive ATPase activity and the lipid peroxidation products. **Results:** After 2 weeks, Na+-K+-ATPase activity was significantly reduced in untreated diabetic rats with respect to that of control groups ($p = 0.03$), whereas an increase of Na+-K+-ATPase activity by 1.2- and 1.8-fold was in 2% and 5% taurine-treated diabetic rats ($p < 0.0001$), respectively. In the latter group, a significant pump hyperfunction was also found at 1 and 2 months with respect to nondiabetic ($p < 0.01$) and untreated diabetic ($p < 0.0001$) rats. Untreated diabetic rats showed a significant progressive reduction of Na+-K+-ATPase activity ($p < 0.01$) with respect to control and the two taurine-treated diabetic rats until the end of the study. At 4 months of diabetes, a normal pump activity was found in the 2% and 5% taurine-treated diabetic rats as compared to nondiabetic rats. CDs and LPs were found to be significantly increased in untreated diabetic rats after 2 weeks ($p < 0.001$); CDs increased in 2% and 5% taurine-treated rats after only 2 month ($p = 0.0001$). In spite of a significant increase of LPs in untreated diabetic rats after 1 month, we found a normal LPs in 2% treated rats until 2 months and in 5% rats until the end of the study. Significant correlations between CDs and LPs ($p < 0.0001$) and pump activities were found. **Conclusions:** In experimental diabetes sustained hyperglycaemia produces neural cell changes and membrane destabilization in diabetic retinas causing an early Na+-K+-ATPase dysfunction. Dose-dependent supplementation of taurine in diabetes may contribute to preventing the development of neuroretinal abnormalities.

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RETINAL VESSEL REACTIVITY IS ALTERED IN ADULT RATS FED PRE AND POSTNATALLY WITH A LOW PROTEIN DIET.

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Background: Glucose intolerance, diabetes and cardiovascular diseases may be consequences of early life events. Abnormal vascular reactions to hypoxia occur in offspring from ewes on caloric restriction. Lower insulin secretion with lower islet and brain vascularization were observed in adult rats fed pre-and postnatally with an isocaloric low protein (LP) diet (8% vs 20%). **Aim**: This study aims at identifying vascular alterations in the retina of LP rats in order to recognize the specific role of protein during vascular development. **Materials and Methods:** A contact lens was placed on the cornea to view the fundus with an operating microscope. A laser beam was directed at a retinal vein for laser Doppler flowmetry. Anesthetized rats were cannulated and ventilated with air followed either by 100% O_2 or air+5% CO_2. **Results:** In female rats, the basal diameter of arteries and veins as well as the basal venous velocity were smaller in the LP rats ($p<0.01$). In the latter, arterial vasoconstriction and blood velocity changes following hyperoxia were not observed in LP (diameter: -1.3% in LP, n=7, vs -9.5% in C, n=7, $P<0.01$; velocity: -3% in LP vs -22% in C, $P<0.05$). Hypercapnia increased venodilation in LP vs C ($p<0.01$). In male rats, the basal diameter of arteries was larger in the LP rats than in C rats ($p<0.05$). Reaction to hyperoxia was similar in C and LP but hypercapnia induced a smaller increase of both arterial diameter (+11% in LP, n=8, vs +26% in C, n=8, $P<0.05$) and blood velocity (+2% in LP vs +22% in LP, $P<0.05$) in the LP compared to C rats. **Conclusions:** These results indicate that LP diet alters retinal vascular reactivity differently in male than in female. They suggest that protein deficiency during pre-and posnatal life could contribute to the development of early retinal abnormalities which may be observed also in diabetes.

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DIABETIC MICROANGIOPATHY AND 4G/5G POLYMORPHISM OF THE PAI-1 GENE PROMOTER IN TYPE 1 DIABETIC PATIENTS.

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Aims: The 4G/5G polymorphism in the promoter of the PAI-1 gene has been related to plasma levels of PAI-1, the main inhibitor of fibrinolysis. PAI-1 level is higher in IDDM patients with microalbuminuria. **Materials and Methods:** The relation between 4G/5G polymorphism and diabetic microangiopathy was investigated in 375 type 1 patients (age 16-69, duration >1-45 years); 94 healthy subjects act as controls. The 4G/5G genotype was evaluated by polymerase chain reaction and endonuclease digestion. Nephropathy was assessed by AER (three-24-hour urines): normal AER (<20 µg/min) 63%; microalbuminuria (20-200) 24%; overt nephropathy (>200) 13%. Retinopathy was assessed by ophthalmoscopy and retinal photography and classified as no retinopathy (25%), nonproliferative (45%) and proliferative (30%). **Results:** 4G/4G, 4G/5G and 5G/5G were in 22.3, 59.6 and 18.1% of controls (Hardy-Weinberg equilibrium: $\chi^2=3.55$, p=0.17) and in 27.5, 49.1 and 23.5% of IDDM ($\chi^2=0.11$, p=0.95). The prevalence of microalbuminuria was 27.7, 24.4 and 18.5 in 4G/4G, 4G/5G and 5G/5G, respectively, that of overt-nephropathy was 17.0, 11.9 and 11.1% ($\chi^2=4.68$, p=0.32). Prevalence of raised AER increases moving from 5G/5G (29.6%) throught 4G/5G (36.3%) to 4G/4G (44.7%, p=0.11). The prevalence of nonproliferative retinopathy was 38.1, 46.7 and 48.7 in 4G/4G, 4G/5G and 5G/5G, respectively, that of proliferative was 40.2, 29.1, and 22.5%. ($\chi^2=7.03$, p=0.13). Prevalence of proliferative retinopathy was higher in subjects with the 4G allele (35.1 vs 25.8%, p=0.03) and in 4G/4G (40.2%) than in 4G/5G+5G/5G (26.9%, $\chi^2=5.75$, p=0.016). Significance remains after controlling for age, BMI, diabetes duration, HbA1c and AER levels. The odds ratio for retinopathy in 4G/4G compared with 4G/5G+5G/5G was 1.82 (95% CI: 1.11-2.99). **Conclusions:** These findings indicate that in caucasian type 1 diabetics, presence of the 4G/4G genotype is associated with a higher risk of proliferative retinopathy, but not of with a higher risk of raised AER.

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PERICYTE RECRUITMENT AND ANGIOGENESIS ARE ALTERED IN HETEROZYGOUS PDGF-BB KNOCKOUT MICE

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Aim: Pericyte recruitment is an important final step in the maturation of the vascular network, and is determined, among others, by the activity of the PDGF-BB/PDGF-ß receptor system. Mice with a homozygous deletion of the PDGF-BB gene die around birth from cerebral hemorrhages and show pericyte deficiency and microaneuryms in brain capillaries. Pericyte loss plays a possible role in the progression to proliferative diabetic retinopathy. We studied the impact of defective pericyte function on retinal morphology and the propensity to develop retinal neovascularizations using heterozygous (+/-) PDGF-BB knockout mice.

Methods: Pericytes numbers from wildtype (WT; C57Bl/6) and +/- PDGF-BB mice (determined by PCR) were evaluated by quantitative morphometry of PAS-stained retinal digest preparations. The presence of pericytes in the deep capillary network was examined by immunohistochemistry using a PDGF-ß receptor antibody. Reactive neovascular response was studied in the model of oxygen-induced retinal neovascularization.

Results: Adult PDGF-BB +/- mice had 28 % fewer pericytes compared with wildtype mice (PDGF-BB +/- 1680±140 cells/mm^2 of capillary area vs WT 2330±75 cells/mm^2; p=0.0011). Qualitatively, the number of PDGF-ß receptor positive capillary profiles in the inner nuclear layer was reduced in PDGF-BB+/- mice compared with WT. The neovascular response to hyperoxia-induced vascular occlusion and reactive ischemia was increased by 85 % in PDGF-BB +/- mice, compared with normal mice (PDGF-BB +/- 47±4.9 vs WT 25.4±2.9 neovascular nuclei /section; p= 0.03).

Conclusions: The PDGF-BB/PDGF-ß receptor system is involved in the recruitment of pericytes in the normal mouse retina, and plays an important role in the modulation of the postnatal neovascular response.

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7-OXO-DEHYDROEPIANDROSTERONE PREVENTS HIGH GLUCOSE INDUCED BOVINE RETINAL CAPILLARY PERICYTES LOSS.

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Aims: The loss of retinal capillary pericytes is an early feature of diabetic retinopathy and is considered to represent a key step in the progression of this complication. We recently reported that, *in vitro*, dehydroepiandrosterone (DHEA) exerts a protective effect on high glucose-induced toxicity in bovine retinal capillary pericytes (BRP). This effect is shown at concentrations equal or more than 100 nmol/l, does not involve the androgen or the estrogen receptors and is thought to be due to the antioxidant properties of DHEA. Nevertheless, the hazards related to the metabolization of DHEA into male or female sex hormones partially limit its clinical use. Aim of this work is to investigate if the DHEA derivative 7-oxo-DHEA, which cannot be converted to either androgens or estrogens, is also capable of protecting BRP against glucose toxicity. **Materials and Methods:** BRP were cultured in either 5.6 or 30 mmol/l glucose, with or without 7-oxo-DHEA at four different concentrations (10, 50, 100, 500 nmol/l). After 4 days of culture, cells were harvested and counted. Statistical comparison between groups was carried out by paired data two-tailed Student's t test. **Results:** The number of BRP grown in glucose 30 mmol/l was significantly lower than that of BRP cultured in glucose 5.6 mmol/l (-31.6%; p< .01). The addition of 7-oxo-DHEA to the culture medium, at all the concentration tested, shielded BRP number from the high glucose effect: 7-oxo-DHEA, already at a concentration of 10 nmol/l, completely reverted the effect of high glucose on cell number (-2.8% vs glucose 5.6 mmol/l; +42% vs glucose 30 mmol/l, p< .05). The growth of BRP in normal glucose concentrations (5.6 mmol/l) was not affected by 7-oxo-DHEA. **Conclusions:** Data show that 7-oxo-DHEA protects BRP against glucose-toxicity. This effect is similar to that displayed by DHEA, but it is exerted at concentrations ten times lower. These data suggest that 7-oxo-DHEA has the same positive and potentially useful properties of DHEA, being devoid of risks related to the conversion to androgens or estrogens.

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Impacts of angiotensin I converting enzyme inhibitor on retinal hemodynamics in type 2 diabetic Chinese

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The purpose of the study is to investigate the effects of angiotensin I converting enzyme inhibitor(ACEI) on the abnormalities of retinal hemodynamics in type 2 diabetic Chinese.105 type 2 diabetic Chinese of preclinical retinopathy stage recruited and randomly assigned two groups: with (female /male=28/36) and without (female/male=17/24) captopril therapy. The degrees of diabetic control were consistent between groups. Peak systolic velocity (PSV), end-diastolic volume (EDV) and acceleration (A) of the central retinal artery (CRA), as well as the reflux rate of central retinal vein (CRV) were measured by ultrasonography. Meanwhile,24h ambulatory blood pressure monitoring, HbA1c and serum lipid profile were determined at baseline, 3 and 6 months in all subjects. Significant differences were found in PSV, EDV, A and CRV of both eyes between two groups not only at the end of 3 months (P=0.0001, 0.0001, 0.0240 and 0.0030 respectively in the left eyes, P=0.0012, 0.0006, 0.0385 and 0 0001 respectively in the right eyes), but also at the end of 6 months (P=0.0101, 0.0003,0.0022 and 0.0006 respectively in the left eyes, P=0.0050, 0.0048, 0.0003 and 0 0020 respectively in the right eyes). The systemic blood pressure and HbA1c, as well as serum lipid profile were not significantly changed before and after captopril administration.In conclusion, ACEI (captopril) could significantly improve the abnormalities of retinal hemodynamics in type 2 diabetic Chinese, but it still needs further study whether ACEI can prevent the progress of retinopathy.

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Macroangiopathy

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MACROVASCULAR COMPLICATIONS AND DEATH FOLLOWING FIRST MYOCARDIAL INFARCTION IN TYPE 2 DIABETES: A POPULATION-BASED STUDY

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One of the key objectives of the St Vincent Declaration is to reduce morbidity and mortality from cardiovascular disease, yet there are few population based data on the incidence and outcome of acute myocardial infarction (AMI) in type 2 diabetes (DM2). **Aims:** To determine the incidence of death and macrovascular complications after a first AMI in people with DM2. **Methods:** Using the DARTS/MEMO database of all diabetic patients in Tayside (population 364880; 7079 DM2, prevalence 2.26%) Scotland, we performed a retrospective prevalence cohort study of all subjects hospitalised with a diagnosis of first AMI from April 1st 1993 to 31st December, 1994. The primary endpoint was time to death; secondary endpoints were two year incidence of angina, AMI, stroke, CCF, CABG and PTCA. **Results:** A total of 2028 patients were admitted with first AMI; 237 (11.6%) were patients with DM2 (incidence 1,913 per 100,000 patient years). Patients with DM2 had worse survival with an increase in relative hazard of 67%. After adjustment for age, sex, smoking, hyperlipidaemia and hypertension, DM2 patients had a 50% higher death rate (p=0.022). There was no significant increase in death rates between diabetic patients and non-diabetics in those >70 years.There was evidence of a trend with age: those diabetic patients aged <60 years had a 4.09 (95% CI 1.10-15.26; p=0.036) increase in death rate; those aged 61-70 a 2.62 (95% CI 1.21-5.68; p=0.015) increase in death rate. **Conclusions:** This population-based study has set targets for the implementation of the St Vincent Declaration. Amongst hospitalised patients with first AMI, diabetes was consistently associated with increased mortality.The increased mortality is most pronounced in DM2 patients aged< 60 years.

1202

MICROALBUMINURIA PLUS RETINOPATHY : A RELIABLE INDEX OF SILENT MYOCARDIAL ISCHEMIA IN TYPE 2 DIABETICS

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Coronary Artery Disease (CAD) constitutes a major complication in type 2 Diabetes Mellitus (DM2) and often is asymptomatic (silent myocardial ischemia) revealed only by specific investigations. Tl-201 tomographic myocardial scintigraphy (Tl) considered as a reliable method for CAD diagnosis is expensive, laborious and radioactive. Microalbuminuria (MA) constitutes an independent risk factor for CAD development in DM2. Diabetic retinopathy (DR) occurs more frequently in diabetics with MA. **Aims :** To investigate whether MA and/or DR can be used as reliable indices of CAD in asymptomatic patients with DM2. **Materials and Methods :** Forty five type 2 diabetics (32 males and 13 females, aged 59 ± 6.8 years and known diabetes duration 9.5 ± 8.2 years) without any clinical or electrocardiographic evidence of CAD. Tl was performed in all diabetics and was used as a reference method for CAD diagnosis. MA was determined by RIA (8h overnight urine collection, nv: <20μg/min) and HbA1c by HPLC. DR was evaluated by fundoscopy. For the statistical analysis, Chi square and Mann Whittney tests were performed. **Results :** It was found that 14 of the 45 diabetics, were Tl positive for CAD. No difference was found between Tl positive and Tl negative for CAD, in age, diabetes duration, blood pressure, BMI, HbA1c and serum lipids. In revealing CAD the coexistence of MA and DR displayed: sensitivity 0.78 (7 / 9), specificity 0.94 (17 / 18), Youden index 0.72, positive predictive value 0.88 (7 / 8) and negative predictive value 0.90 (17 / 19); thus false positive and false negative findings were about 10%. **Conclusions :** The above data indicate that the evaluation of MA and DR constitutes a simple and highly reliable method for CAD diagnosis, even during an asymptomatic stage, giving thus, the opportunity for therapeutic interventions.

1203

CARDIOVASCULAR DISEASE IN PATIENTS WITH HYPERTENSION AND DIABETES MELLITUS IN THE NORTH OF PORTUGAL

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Aims: To evaluate the incidence of cardiovascular disease (CVD) in hypertensive (HT) patients with and without diabetes mellitus (DM) admitted to a central hospital in the North of Portugal, between 1989 and 1998. **Materials and Methods**: We retrospectively analysed data from all HT patients aged 25 years or older who satisfied the International Classification of Diseases (9th version) criteria for CVD (ICD9 codes 390–459). Statistical analysis was performed with Student's t-test, χ^2 test or Fisher exact test. A two-tailed p value < 0.05 was considered significant. **Results:** HT patients with DM [36.8% men (M) and 63.2% women (W)] were significantly older than HT patients without DM (57.0% W and 43.0% M) (65.2±1.1 vs 63.1±1.4 years, $p<0.01$). There were no significant differences in duration of hospitalization in HT patients with and without DM (14.3±0.93 vs 13.54±1.05 days). The incidence of fatal and nonfatal cardiovascular events was 38.0% (n=2886) in HT patients with DM and 35.4% (n=11006) in HT patients without DM. HT patients with DM had an incidence of coronary heart disease (9.8%), cardiac heart failure (6.0%), and peripheral vascular disease (2.2%) that was significantly different ($p<0.01$) from that in HT patients without DM (8.9%, 4.4%, 1.3%, respectively). There were no significant differences in the incidence of cerebrovascular disease in HT patients with and without DM (20.4% vs 20.8%). There was an excess of fatal myocardial infarction among the HT patients with DM, but this was not significant (13.3% vs 10.8%). **Conclusions:** The high incidence of CVD in patients with hypertension and DM imply that primary and secondary preventive strategies should become an integral part of their medical care, with a delineation and acceptance of responsibilities between the primary care physicians and diabetes specialists.

1204

ABSENCE OF CHLAMYDIA PNEUMONIA IN THE ATHEROMATOUS PLAQUES OF TYPE 2 DIABETIC PATIENTS

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AIMS: The present study has been designed to demonstrate the presence of Chlamydia Pneumonia infection in the atheromatous plagues of the patients with Type 2 diabetes mellitus.**MATERIALS AND METHODS:** A total of 30 artery segments from 15 type 2 diabetic and 15 nondiabetic subjects were examined. Using polymerase chain reaction, Chlamydia Pneumonia DNA was looked for in the surgically excised atheroma specimens of the patients obtained from coronary bypass grafting (n=12) and carotid endarterectomies (n=18). **RESULTS:** The diabetic and nondiabetic groups were matched with respect to age, body mass index, history of smoking and serum lipid parameters. The mean duration of diabetes mellitus was 7.8 ± 3.2 years for the diabetic group and the mean Hb A_{1c} level was 7.2 ± 1.2 % for the diabetic group. Chlamydia Pneumonia antibodies were determined by immunofluorescence technique and revealed a similar Ig G seropositivity in both groups (3/15 and 4/15 for the diabetic and the nondiabetic group respectively). None of the specimens were positive for Chlamydia Pneumonia DNA by polymerase chain reaction. **CONCLUSIONS:** Results of this study suggest that diabetic patients with atherosclerosis do not have an increased incidence of Chlamydia Pneumonia infection with regard to nondiabetic population and probably Chlamydia Pneumonia is not a significant factor in the pathogenesis of atherosclerosis in the diabetic patients.

1205

MAJOR DETERMINANTS OF THE CAROTID INTIMA-MEDIA THICKNESS IN TYPE 2 DIABETIC PATIENTS: AGE AND BODY MASS INDEX

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AIMS: The present study has been designed to quantify and compare right and left carotid intima-media thicknesses seperately in type 2 diabetics and healthy controls. It was also intended to investigate the effects of various risk factors on the carotid intima-media thickness in these subjects. **MATERIALS AND METHODS:** A total of 122 subjects; 70 patients with type 2 diabetes and 52 non-diabetic subjects as controls, were recruited for the study. Right and left common carotid artery stiffness indices were assesed with noninvasive ultrasound method in both groups. Age, body mass index, duration of diabetes, cigarette smoking, lipid profile including lipoprotein a, Chlamydia pneumonia seropositivity, glycemic indices, fasting insulin levels, serum fibrinogen levels and presence of hypertension, coronary artery disease, degenerative complications of diabetes mellitus were all assessed in order to define their role as determinants of carotid artery intima-media thickness. **RESULTS:** The difference between the groups regarding mean intima-media thickness was statistically significant for the left carotid arteries ($p=0028$) and borderline significance was found for the right carotid arteries ($p=0.055$). Age has a very strong association with carotid intima-media thickness in diabetic patients ($p<0.0001$) with univariate analysis. According to the results of multiple regression analysis, age and BMI were found to be the most important independent determinants of carotid intima-media thickness for both sides. When age was excluded from the model, BMI and coronary artery disease were found to have strong association with intima-media thickness on the right ($p=0.0036$ and 0.0249) and BMI was the only significant determinant for the left side ($p=0.0025$). **CONCLUSIONS:** This study shows that carotid artery intima-media thickness, especially on the left side, is greater in diabetic subjects compared with healthy controls. For the diabetic subjects age, BMI and presence of coronary heart disease has strong influence on the atherosclerotic process of the carotid arteries.

1206

LEUCINE 7 TO PROLINE 7 POLYMORPHISM IN THE NPY GENE IS ASSOCIATED WITH INCREASED CAROTID ATHEROSCLEROSIS

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Neuropeptide Y (NPY) is a 36-amino-acid neurotransmitter widely present in the central and peripheral nervous systems. NPY has multiple actions, which control body energy balance and cardiovascular function. We have recently demonstrated that subjects having Pro7 in the signal peptide of NPY have higher serum cholesterol and apolipoprotein B levels when compared to individuals having wildtype (Leu7/Leu7) signal peptide sequence (Nat Med 1998;4:1434-7). **Aim**: To investigate the association of Leu7Pro polymorphism of the NPY gene to quantitative atherosclerosis. **Subjects and Methods:** Subjects were newly diagnosed patients with Type 2 diabetes (81 patients, 41 males, mean age 67.1 years) and non-diabetic subjects (105 subjects, 48 males, mean age 65.5 years) genotyped for the Leu7Pro-polymorphism in preproNPY at the 10-year follow-up study. The common carotid intima-media-thickness (IMT) was assessed by ultrasonography. **Results:** The frequency of the Pro7 in preproNPY was 9.9% in diabetic patients and 14.3% in control subjects ($p=0.360$). The mean common carotid IMT was in non-diabetic subjects without the Leu7Pro polymorphism 1.04±0.02 and with it 1.14 ± 0.04 mm ($p=0.156$) and in diabetic patients 1.18±0.03 and 1.58±0.21 mm ($p=0.004$), respectively. In the analysis of covariance of the entire group the mean common carotid IMT was independently associated with the Leu7Pro-polymorphism ($F=5.165$, $p=0.024$). The model included also age, gender, diabetes, clinical macrovascular disease, smoking, systolic blood pressure and LDL-cholesterol. **Conclusions:** The presence of the recently described Pro7 substitution in the preproNPY associates with increased carotid atherosclerosis in diabetic and non-diabetic subjects, even after adjustment for known risk factors.

1207

ANKLE/BRACHIAL INDEX IS A PREDICTOR OF CARDIOVASCULAR DISEASE, INDEPENDENT OF MEDIASCLEROSIS.
The Pittsburgh Epidemiology of Diabetes Complications (EDC) Study.

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Aims The validity of the ankle/brachial systolic blood pressure index (ABI) has been questioned in patients with diabetes because of media wall calcification, which would falsely raise the ankle pressure and ABI. The gold standard for mediasclerosis is the presence of calcification in the arteries on X-ray. We performed a prospective study to determine the risk of ABI and mediasclerosis for cardiovascular disease over 6 years.
Materials and Methods The study was conducted within the EDC Study, a follow-up study based on an incident cohort of children with diabetes type 1. During the third follow-up examination X-rays of the foot and ankle were made. Calcification was defined by specific outlines of continuous calcifications in at least one artery. The study population consists of all patients with calcification (n=50) and 50 patients without calcifications, matched for diabetes duration. Cardiovascular disease (CVD) at the last follow-up examination is defined as death, myocardial infarction, CVA or peripheral vascular disease. All analyses are adjusted for gender and duration of diabetes.
Results The mean follow-up period was 5.9 years (SD 0.5). At the time of the X-rays the age of the study population (52 men) was 35.6 years (6.3), mean duration of diabetes 27.5 years (6.6), and the mean lowest ABI 1.07 (0.14). At the last follow-up examination CVD was present in 23%. Calcification was not associated with an increased risk of CVD: odds ratio 0.61 (95% CI 0.21-1.81). The odds ratio of peripheral artery disease (ABI<0.9 or occlusion) was 2.80 (0.87-9.07). In patients with calcifications this was 3.76 (0.82-17.3), and in those without 5.86 (0.38-89.4). The odds ratio of ABI≥1.3 was 1.69 (0.60-4.81). In patients with calcifications this was 0.94 (0.20-4.36), and in those without 5.11 (0.98-26.8). Calcification was also not associated with decreased toe/ankle blood pressure index (p>0.3).
Conclusion Low ABI (<0.9) is probably a predictor of cardiovascular disease, independent of calcification on foot X-ray. Mediasclerosis defined by an ABI≥1.3 is a predictor of clinical CVD in patients with calcifications only.

1208

Reduced ankle brachial index is strongly linked to albumin excretion rate and white cell count but not to measures of endothelial dysfunction or coagulation in NIDDM

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Aims: There is limited information on the relationship between peripheral vascular disease and microalbuminuria in NIDDM. We have studied the relationships between reduced ankle brachial pressure index (ABI) and measures of coagulation, fibrinolysis and endothelial dysfunction in NIDDM patients with and without microalbuminuria.
Materials and methods: NIDDM patients with no history of coronary disease or heart failure were studied. Forty-three patients with microalbuminuria were matched with 43 normoalbuminuric patients (albumin excretion rate (AER) [median (range)] 28 (11-164) vs 4 (2-9) mg min^{-1}, p<0.001) for age, gender, diabetes duration and smoking status. Ankle systolic pressure was measured using a doppler ultrasound technique and brachial systolic pressure by the auscultatory method. **Results**: ABI was reduced in the microalbuminuric group (1.06 vs 1.14, p=0.03). Plasminogen activator inhibitor$_1$ (115 vs 100 $ng.ml^{-1}$, p=0.001), thrombin antithrombin complex (5.1 vs 3.4 $\mu g.l^{-1}$, p=0.03), E Selectin (65 vs 52 $ng.ml^{-1}$, p=0.009), Factor XIIa (3.2 vs 2.3 $ng.ml^{-1}$, p=0.006) and fibrinogen (4.1 vs 3.6 $g.l^{-1}$, p=0.01) were all higher in the microalbuminuric group. White cell count was similar in both groups (6.7 vs 6.3 $x10^9.l^{-1}$, p=0.18). In combined microalbuminuric and normoalbuminuric groups, reduced ABI was significantly related in univariate analysis to white cell count (r=0.29, p=0.007), AER (r=0.28, p=0.011), HDL cholesterol (r=-0.26, p=0.022), $\log_e$ fibrinogen (r=0.25, p=0.024), triglyceride (r=0.24, 0.033) and factor XIIa (r=0.23, p=0.036). There were no significant relationships with age, gender, blood pressure, smoking status, BMI or glycaemic control. In stepwise multivariate analysis only white cell count (t=2.56) and AER (t=2.44) showed significant independent relationships with reduced ABI. **Conclusions:** Reduced ABI does not show independent relationships with measures of coagulation, fibrinolysis and endothelial dysfunction. Microalbuminuria may be a useful marker of peripheral vascular disease in NIDDM. The link between reduced ABI and raised white cell count is consistent with the inflammation/infection hypothesis of atherosclerosis.

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Circulating Indicators of Endothelial Dysfunction

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LEVELS OF SERUM CELL ADHESION MOLECULES IN TYPE I DIABETIC PATIENTS WITH MICROANGIOPATHY

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Cell adhesion molecules seem to play an important role in the development of late vascular diabetic complications. **Aims:** the aim of the study was to evaluate concentration of selected serum cell adhesion molecules in Type I diabetic patients. **Material and Methods:** the study was performed in 20 patients with retinopathy and nephropathy (group A: aged 27.2±10.4 years, 9 female and 11 male, duration of disease 16.2±6.2 years and HbA1c 8.3±2.7 %) and 20 patients without late diabetic complications (group B: aged 26.4±6.8 years, 10 female and 10 male, duration of disease 7.1±4.6 years and HbA1c 8.0±2.6 %). Soluble form of Intercellular Adhesion Molecule-1 (ICAM-1), Endothelial Leukocyte Adhesion Molecule-1 (E-selectin) and Vascular Cell Adhesion Molecule-1 (VCAM-1) levels were estimated with the use of the ELISA test. **Results:** in comparison with healthy subjects we observed significantly higher values of ICAM-1 and VCAM-1. VCAM-1 level in group A was markedly higher in comparison with group B. We did not observe statistically significant differences in the other parameters. The results are shown in a table:

parameter	Healthy subjects	Group A	Group B
sICAM-1 (ng/ml)	210.60±7.16	244.98±9.70 *	229.73±8.38 *
sVCAM-1 (ng/ml)	553.02±10.50	728.41±30.76 * †	602.53±27.85 *
E-Selectin (ng/ml)	46.25±1.18	47.77±3.89	48.91±3.37

* p<0.05 diabetic patients vs healthy subjects, † p<0.05 group A vs group B

Conclusions: the results might suggest that disturbances in cell adhesion molecules levels in diabetes are independent from microangiopathy.

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PLASMA LEVELS OF TISSUE SPECIFIC-FIBRONECTIN IN DIABETES

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Aims: Tissue specific-fibronectin is an endothelium derived protein involved in subendothelial matrix assembly. Elevated plasma levels of tissue specific-fibronectin therefore reflect loss of endothelial polarization, ie injury to blood vessels. We investigated whether tissue specific-fibronectin may be a useful marker of the specific changes in patients with diabetes mellitus associated with vascular damage.
Methods: We performed a cross-sectional study, involving 73 patients with diabetes (23 type 1 and 50 type 2) at high risk for diabetic angiopathy and 29 type 1 diabetic patients without micro- or macrovascular complications. Reference groups consisted of 64 healthy persons, 54 ischemic stroke patients and 23 patients with renal artery stenosis. Tissue specific-fibronectin was measured in plasma of these subjects.
Results: Circulating tissue specific-fibronectin was significantly elevated in the high risk group of patients with diabetes (5.2 ± 2.8 µg/ml) compared with patients with diabetes without complications (2.1 ± 1.1 µg/ml), with ischemic stroke (2.0 ± 0.9 µg/ml), renovascular hypertension (1.7 ± 1.1 µg/ml) and healthy subjects (1.4 ± 0.6 µg/ml). This was independent of other patient characteristics.
Conclusion: Plasma tissue specific-fibronectin may be a useful marker protein to detect endothelial damage in well-defined groups of patients. It may not be a general marker for vascular disease, but seems to point at a specific diabetes associated process of angiopathy .

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EVALUATION OF ELASTIN METABOLISM-A LONGITUDINAL STUDY IN CHILDREN WITH TYPE 1 DIABETES MELLITUS
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Serum elastin antibodies(EA) and elastin-derived peptides(EDP) appear to correlate with breakdown of elastic tissue. For the presence of EA (IgG, IgM and IgA) and EDP were tested by ELISA 28 children with Type 1 diabetes mellitus, without any clinical and laboratory data for vascular complications, during 6 years (mean age 11.4±2.8 years, diabetes duration- 5.5±1.9 years). Twenty four healthy children of similar age and sex served as a control group. During the first two years the majority of patients were positive for EA of the IgM type (11%), during the second- for IgG (14%) and during the third-for IgA(32%). The concentration of EDP was significantly increased ($p<0.05$) during the first two years in 11%, during the second in 14% and during the third in 21% of the patients. At the end of the investigated period 6 (21%) diabetic children develop vascular complications. All of them were with enhanced elastin metabolism. The elevation of IgM and IgG may suggest the initial stage of the autoimmunization to elastin while the elevation of IgA and EDP more increased elastin degradation and development of vascular complications. In conclusion, the detection of early changes in the levels of EA and EDP would allow to make an early diagnosis of diabetic vascular complications.

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The effect of smoking on transforming growth factor-ß levels and lipid peroxidation in diabetes mellitus

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Smoking is a risk factor for the development of diabetic nephropathy and retinopathy. In addition, oxidative stress and cytokines have been related to the occurrence of diabetic micro and macrovascular complications. **Aim**: To analyze the effect of smoking on plasma 8-epi-$PGF_{2\alpha}$ levels, as a index of lipid peroxidation and transforming growth factor-β (TGF-β), as potent inducer of extracellular matrix production and fibrogenesis, in diabetic patients. **Patients and methods**: 16 type 1 diabetic patients, 8 non-smokers and 8 smokers (>15 cigarettes/day), matched for age, diabetes duration, BMI and HbA_{1c} were studied. As the control group (CG) 8 non-smokers normal subjects were included. Activated TGF-β and total 8-epi-$PGF_{2\alpha}$ were measured by enzyme immunoassay.

Results:	*smokers*	p	*non-smokers*	p	*CG*
n:	8		8		8
Age (yr.)	34.6±4.5		30.3±7.1		35.5±6.9
Sex (male%)	50		40		62
Diabetes duration (yr.)	10.6±5.1		12.7±4.4		--
BMI (kg/m^2)	23.1±1.5		22.3±1.8		24.2±1.9
HbA_{1c} (%)	7.0±1.1		7.1±0.9		--
TGF-β (ng/ml)	17.9±6.2	---<.05---	8.6±5.1	---<.05---	4.1±1.6
8-epi-$PGF_{2\alpha}$ (pg/ml)	141±27		177±51		171±40

Considering all the patients together there was no relationship between TGF-β and any of the variables studied. 8-epi-$PGF_{2\alpha}$ plasma levels correlated significantly with the HbA_{1c} ($r=0.55$, $p=0.03$). **Conclusions**: Diabetes induces an increase in TGF-β plasma levels and this effect seems to be magnified by tobacco consumption This may play a role in the pathogenesis of diabetic complications. glycemic control appears to influence lipid peroxidation in these patients

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IS PLASMA ET-1 A MARKER OF VASCULAR COMPLICATIONS IN TYPE 2 DIABETES MELLITUS?

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Endothelin-1 (ET-1) is a strong vasoconstrictor agent with tissue proliferative properties. ET-1 was suggested to play a role in the development of diabetic vascular disease. The **aim** of our study was to determine circulating ET-1 in well and poorly controlled type 2 diabetic patients with and without vascular complications or hyperlipidaemia. **Materials and Methods:** Circulating plasma ET-1 level was measured by a self-developed radioimmunoassay in 80 type 2 diabetic patients (age: 58,38±9,64 years; sex: 37 male, 43 female; BMI: 29,46±5,25kg/m^2) and in 28 healthy control subjects (age:48,21±5,26 years; sex. 9 male, 19 female; BMI: 26,92±3,51kg/m^2). **Results:** We have found significantly lower circulating ET-1 concentration in type 2 diabetic patients as compared to control subjects (11,74±3,52 vs. 13,24±2,6pg/ml, $p<0,05$). Plasma ET-1 level was similar in control subjects with (n=21) or without (n=7) elevated blood lipid levels (12,31±2,42 vs. 13,55±2,64 pg/ml). There was no difference between control normolipidaemic subjects and diabetic hyperlipidaemic (n=13) patients (12,31±2,42 vs. 12,46±3,42 pg/ml). However diabetic hyperlipidaemic patients (n=67) had significantly lower circulating ET-1 concentration (11,6±3,55 pg/ml) than control hyperlipidaemic subjects (13,55±2,64, $p<0,05$). Diabetic patients without vascular complications (n=52), had significantly lower plasma ET-1 levels than concentrations found in control subjects (11,84±3,19 vs. 13,24±2,6 pg/ml, $p<0,05$). Plasma ET-1 in diabetic patients with complications (n=28) did not differ from control subjects (13,24±2,6 vs. 11,55±4,12 pg/ml). **Conclusion:** In our previous study (EASD 98) with type 1 diabetic patients with vascular complications we have found significantly higher ET-1 as compared to healthy control subjects. The molecular basis of vascular complications might be different in type 1 and 2 diabetes mellitus. We suggest that plasma ET-1 level is not a sensitive marker of vascular complications in type 2 diabetes mellitus. Sponsored by ETT (T 02071, 1997), OTKA (T 025920, 1997) Zsigmond Diabetic Foundation.

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LIPOPROTEIN (a) LEVELS IN FAMILIES OF TYPE 1 DIABETIC PATIENTS IS CORRELATED WITH PROBANDS' DIABETIC COMPLICATIONS.

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Lipoprotein (a) has been identified as an independent cardiovascular risk factor for atherosclerosis. Lp(a) serum concentrations seem to be increased in type 1 diabetes mellitus, but the association of Lp(a) and diabetic complications is still controversial. The highest levels observed in diabetic nephropathy could either predate or follow overt albuminuria. We measured Lp(a) concentrations by immuno-nephelometry in 30 type 1 diabetics (age 35±10 yr, disease duration 21±9 yr; no complications, n=10, retinopathy, n=9, nephropathy, i.e. urinary albumin excretion rate > 20 µg/min, n= 11), their non-diabetic relatives, 47 parents (60±10 yr) and 36 siblings (39±12 yr), and 60 non-diabetic controls without family history of type 1 diabetes.

Median serum Lp(a), but not total or HDL cholesterol and triglyceride levels, were significantly increased in both diabetics (14 mg%, range 3-153), their parents (11, 5-167), and siblings (17, 10-157) than in controls (10, 10-46, $p<0.001$). Serum Lp(a) concentrations, although higher in albuminuric diabetic subjects (31.5, 10-153) in comparison with normoalbuminuric patients (12, 3-117), did not reach statistical significance. However, serum Lp(a) concentrations were significantly increased in parents of type 1 diabetics with nephropathy (31.5, 11-61, $p<0.01$) than in parents of diabetics with retinopathy (11, 5-167) or no complications (11, 10-167).

Our study suggest that 1) levels of Lp(a) are increased in families of type 1 diabetics, especially in presence of diabetic nephropathy; 2) since diabetes mellitus and nephropathy are confounding factors in evaluating Lp(a) role into diabetic complications, it seems useful to study their non diabetic first-degree relatives, being Lp(a) under strong genetic control.

PS 109
Haemostasis

1215

DEFECTIVE PROTEIN KINASE C SIGNALING IN HUMAN PLATELETS IN TYPE 2 DIABETES: HERESY OR FACT?

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Aims: Atherosclerosis is the main cause of macroangiopathy and death in type 2 diabetes (T2D). An imbalance in thromboxane (TXA_2) and prostacyclin (PGI_2) levels occurs in T2D which is associated with platelet activation. Since glucose and lipid metabolism is changed and may alter PKC activity in diabetes we developed a bioassay to investigate the role of PKC in thrombin induced TXA_2 release and to compare the function of the enzyme in patients with T2D and controls. **Materials and Methods:** Human platelets were isolated by differential centrifugation. After preincubation for 1 h at 37 °C platelets were stimulated with 0 to 2 U thrombin for 5 min. 30 min before addition of thrombin platelets were incubated with 1 µM of the specific PKC inhibitor GF109203X (or LY279196) and 10 min before thrombin with 100 nM TPA where mentioned. The stable thromboxane B_2 (TXB_2) was measured in supernatant by a quantitative ELISA. In parallel immune blot analyses of platelet cytosolic and membranous fractions were performed for PKCβ2 to assess activation of PKC. Statistical analysis was done with the Wilcoxon test. **Results:** Thrombin stimulated TXB_2 release in a dose dependent manner in T2D and controls. 1 U thrombin elevated TXB_2 release in T2D 6-fold and in controls 2-fold (n=10). Thus TXB_2 release was elevated in diabetes ($p<0.05$). Preincubation with GF109203X stimulated thrombin induced TXB_2 release further. Platelets of type 2 diabetic persons and controls showed an increase of TXB_2 to 290 ± 50 % and 510 ± 70 % respectively relative to platelets stimulated with 1 U thrombin alone (n=12). TPA reduced TXB_2 release in controls more than in T2D ($p<0.05$). Immune blots showed activation of PKC by thrombin. **Conclusions:** Unexpectedly thrombin induced thromboxane release was inhibited by activation of PKC as shown by the increase caused by inhibition of PKC. In T2D this control appears to be ineffective. This is suggested by the smaller response to inhibition of PKC and by the elevated TXB_2 response to thrombin. Together this fits the concept of a role of PKC in atherosclerosis but, in contrast to current theories, suggests that loss of PKC function may be important rather than its overactivation at least with regard to platelets.

1216

PLATELET FACTOR 3 IN PLASMA FRACTIONS OF DIABETIC PATIENTS.

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Platelets take part in haemostasis cascade by the formation of catalytic surface for prothrombinase complex. This procoagulant activity depends on the amount of platelet factor 3 (PF3), which activity is connected with phospholipid particles.
Aims: The aim of our study was the estimation of PF3 activity in platelet rich plasma (PRP), platelet poor plasma (PPP) and filtrated plasma (PFP) of diabetic patients.
Material and methods: The group studied consisted of 13 type 1 diabetics, 23 type 2 diabetics and 18 control subjects. The activity of PF3 was measured using Russell's viper venom (RVV, Sigma Chemicals), activating factor V and X, which – in the presence of $Ca^{2+}$and phospholipids (PF3 activity) – form prothrombinase complex. ANOVA and Mann-Whitney test were used for statistical analysis.
Results: PF3 activity was increased both in type 1 ($p<0.05$ for PPP and $p<0.05$ for PFP) and type 2 ($p<0.05$ for PRP, $p<0.01$ for PPP and $p<0.005$ for PFP) diabetics in comparison with the control group. The highest difference was observed in PFP, reflecting an increased contribution of the smallest microvesicles released from platelet membranes in the formation of catalytic surface for prothrombinase complex. No correlation between PF3 activity and patients age, HbA1c, lipid concentrations nor microalbuminuria were found. However, a correlation between PF3 and fibrinogen level was observed (PRP: $r=0.8434$, $p<0.05$, PPP: $r=0.8721$, $p<0.01$, PFP: $r=0.9115$, $p<0.005$).
Conclusions: 1) Platelet procoagulant activity is increased both in type 1 and type 2 diabetics. 2). Our results suggest an increased contribution of the smallest microvesicles (< 0,1 µm) released from platelet membranes in the activation of haemostasis in diabetic patients.

1217

IS THROMBIN GENERATION ENHANCED IN DIABETIC PATIENTS WITH RETINOPATHY?

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The role of hypercoagulability in the pathogenesis of diabetic retinopathy is still a matter of debate. **Aim:** The purpose of this study was to evaluate the thrombin generation as a hallmark of hypercoagulable state in type 1 diabetic patients. **Materials and methods:** Fifty well-controlled type 1 diabetic subjects (28M: 22F) aged 33.4±9.4 yr with mean diabetes duration 9.5±5.4 yr and 20 well-matched healthy individuals aged 32.3±5.2 yr were selected for this study. Of diabetic patients 22 showed retinopathy (6 proliferative) and 28 were free from any diabetic complications. Routine coagulation parameters, as well as antithrombin III (ATIII), thrombin-antithrombin III complexes (TAT) and D-dimers were assessed in all subjects. Thrombin generation test was carried out according to Kaulla and Biggs in platelet rich- and platelet-poor defibrinated plasma using Fibrintimer (Behring). Thrombin generation was monitored over 15 min with 1 min intervals and related to standard thrombin concentrations curve. **Results:** Patients with retinopathy showed significantly elevated maximal amount of generated thrombin in platelet-rich plasma compared to controls (12.8 U/ml *vs* 9.6 U/ml, $p<0.01$); the other inter-group comparisons were beyond statistical significance. Likewise, no significant differences between groups were revealed for platelet-poor plasma. In patients with diabetic retinopathy the generation of thrombin in platelet-rich plasma correlated positively with plasma cholesterol ($r=0.45$,$p<0.05$) and with diabetes duration ($r=0.47$, $p<0.05$). In diabetics without retinopathy we observed a positive correlation between thrombin generation in platelet-poor plasma and prothrombin time ($r=0.43$, $p<0.05$). The amounts of generated thrombin were not related to ATIII, TAT or other haemostatic parameters in the studied patients. **Conclusions:** Enhanced thrombin generation in platelet-rich plasma in diabetic patients with retinopathy and no differences in platelet-poor plasma may point to the enhanced procoagulant activity of platelet membrane phospholipid bilayer in diabetic patients with retinopathy, which may be one of the major contributing factors to hypercoagulable state in diabetic microangiopathy.

1218

DIFFERENT TRANSLOCATION OF PROTEINKINASE Cß2 IN THROMBIN STIMULATED-HUMAN PLATELETS IN PATIENTS WITH TYPE 2 DIABETES

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Aims: Increased platelet activity, increased intravascular thrombin generation reduced fibrinolytic potential are leading to increased risk of thrombotic complications in diabetic patients. At least some actions of thrombin are negatively regulated by PKC. Our previous data show that PKC-dependent mechanism may be responsible for these pathologic changes. Materials and Methods: Platelets were isolated from whole blood specimen containing citrate and prostacyclin PGI2 by differential centrifugation and incubated 5 min with 0.5, 1 and 2 U / ml human thrombin. As standard we used signal of actin at 45 kDa. PKCß2 was determined using western blot and laser densitometry in platelets membrane and cytosol from T2D patients (n=10) and non-diabetic controls (n=10). Results: We detected three PKCß2 antibodies-sensitive proteins with molecular weight 80, 82 and 84 kDa. An extensive increase of membrane fraction of PKCß2 as observed 5 min after incubation with 0.5, 1 and 2 U / ml human thrombin or PMA 100nM (n=10). In western blots we detected signals of PKCß2 at 80, 82, 84 kDa. After thrombin stimulation PKCß2 signals at 80 and 82 kDa increased 50% more than in T2D compared to controls (n=10). When platelets were stimulated with 100 nM PMA the translocation of PKC ß2 signals at 80 and 82 kDa elevated 30% more in T2D compared to controls (n=10). There was significant difference between control and T2D. Conclusion. Our results indicate that determination of different PKCß2 subfractions in platelets is possible. Probably the reflect three different forms of PKCß2. Possible explanation could be in vivo stimulation of platelets in diabetics(glucose, osmolality changes, thrombin tec.) or defects in intracellular signaling. We report that PKCß2 signal at low molecular weight is increased in T2D. Submaximali stimuli activate PKC ß2 in platelets of T2D indicating a preactivation of PKC ß2 in this disease most likely related to the increased intracellular calcium, DAG or IP3.

1219

THE EFFECT OF SULODEXIDE ON PLATELET REACTIVITY *EX VIVO*

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Several clinical studies have demonstrated the efficacy of sulodexide, a novel glycosaminoglycan agent with antithrombotic activity, in both the treatment and prophylactics of cardiovascular complications, especially in diabetic patients. **Aims:** The aim of the study was to evaluate the effect of sulodexide (Vessel Due F-Wasserman Sp.A.) on whole blood platelet release reaction and reactivity under ex vivo conditions upon platelet activation with selected agonists. **Materials and Methods:** The study was conducted on a group of healthy volunteers and comprised the comparative investigation of the effects of sulodexide (1 μg/ml and 10 μg/ml), standard heparin (0.1 U/ml, 2.0 U/ml) and LMWH (Fraxiparine - Sanofi Winthrop - 0.1 U/ml). The platelet reactivity was evaluated by means of the whole blood cytometry to monitor platelet reactivity (incubation with 0.15 U/ml thrombin and 2.5 mM GPRP, 4 min; 8 μM TRAP, 4 min and 3 μM ADP, 15 s).The following markers of spontaneous and induced platelet activation were investigated: P-selectin (CD62), GPIbα (CD42b), GPIIb/IIIa (CD61), platelet microparticles and platelet aggregates. **Results:** Sulodexide (1 μg/ml) inhibited the release of P-selectin from intraplatelet α granules induced by thrombin by up to 75.2±18.1% ($p<0.002$), also the fraction of platelet aggregates was much reduced by up to 56.2±9.4% ($p<0.05$). Higher drug concentration did not significantly deepen the above effects. No apparent changes in GPIbα internalisation in the presence of sulodexide were observed. The unfractionated heparin and sulodexide acted very much alike in that both drugs showed similar effects on platelet reactivity, as reflected by the changes in the expression of surface platelet membrane P-selectin. The effect of sulodexide (1 μg/ml) on thrombin inhibition in the presence of ATIII was significantly weaker than that observed with the standard heparin (0.1 U/ml) or Fraxiparine (0.1 U/ml) - 48±18%; 78±8%; 71±6% respectively ($p<0.005$). ***CONCLUSION:*** The results of our study suggest that the effect of sulodexide on platelet reactivity does not entirely depend on the inhibition of thrombin proteolytic activity. It can be speculated that the agent may interfere with thrombin action on its receptor on platelet membranes and/or the interfering with platelet signal transduction.

1220

ROLE OF SERUM GLUCOSE AND INSULIN IN THE PATHOGENESIS OF PLATELET HYPERAGGREGATION IN DIABETIC SUBJECTS

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Aims: The exact biochemical risk factors for platelet activation in diabetes are still unresolved and controversy exists whether platelet dysfunction is a primary or secondary event in diabetes. Frequent association of other risk factors such as obesity, hypertension, hyperinsulinemia and dyslipidemia complicates the evaluation of the role of serum glucose and insulin levels on the development of platelet hyperaggregation in diabetic subjects. To clarify these roles a group of newly diagnosed young Bangladeshi diabetic subjects have been studied who, from previous experience, are known to be nonobese, normotensive, normolipidemic, markedly hyperglycemic and normo- to hypoinsulinemic but nonketotic. **Materials and methods:** Twenty one type 2 (Age in yrs: 25±3: and BMI: 20.89±4.02: M±SD) and 10 fibrocalculus pancreatic diabetic or FCPD subjects (Age in yrs: 22±4, BMI: 16.28±1.7) were studied along with age- and BMI-matched nondiabetic controls without family history of diabetes. Platelet aggregation was measured by optical aggregometry and c-peptide was measured by an ELISA technique. **Results:** Fasting plasma glucose (mmol/l) was considerably high both in type 2 (15.3±5.28, M±SD) and FCPD (19.1±4.22) diabetic groups. FCPD subjects had significantly lower fasting C-peptide (ng/ml, 1.02±0.60, M±SD) compared to Control (2.26±0.81) and type 2 subjects (2.30±1.28) ($p<0.001$).Though the C-peptide value was similar to control subjects in Type 2 patients their C-peptide-glucose ratio was found to be significantly lower (Control: 0.49±0.19 vs Type 2: 0.19±0.160, $p<0.0001$) reflecting a deficient insulin secretory capacity. Percentage aggregation (M±SD) of platelets was 83±11 (by ADP), and 92±12 (by collagen) in type 2, 90±8 (by ADP) and 97±5 (by collagen) in FCPD and 78±10 (by ADP) and 89±11 (by collagen) in Control subjects ($p< 0.001$ in Control vs type 2 and FCPD). Correlation analysis showed a significantly negative value between fasting serum C-peptide and collagen-induced platelet aggregation ($r= -0.793$, $p<0.006$), and a positive value between C-peptide and ADP-induced platelet aggregation ($r= 0.461$, $p<0.035$). **Conclusions:** The data suggest that (a) hyperglycemia *per se* may not lead to increased platelet aggregation in diabetic subjects, and (b) insulin may have a complex and dual role on platelet hyperaggregation possibly with a direct positive effect in the hyperinsulinemic and indirect effect in the hypoinsulinemic range.

1221

ADP AND COLLAGEN SENSITIVITY OF PLATELETS IN TYPE 2 DIABETIC PATIENTS IMPROVES AFTER GLYCAEMIC CONTROL

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Aims: Platelet hyperactivity has been reported in poorly controlled diabetes mellitus. We investigated ADP and collagen sensitivity of platelets in type 2 diabetic patients during poor glycaemic state and after glycaemic control is achieved. **Materials and Methods**: Eleven insulin-treated type 2 patients (7 females and 4males) aged between 39-59 (median 45) were studied. Median disease duration was 5 years. Fasting plasma glucose, serum total cholesterol, triglycerides, serum fibrinogen levels were determined both during poor metabolic control and after restoration of glycaemic control as evidenced by a $HBA_{1c} \leq 7.0\%$. ADP and collagen sensitivity of platelets during poor control and after restoration of glycaemic control with Chrono-log 500-VS aggregometer. Platelet aggregation was studied at 4 different final concentrations of ADP (1, 2, 3 and 5 μM) and collagen (0.4, 0.8, 1.2 and 2 mg/ml).Reagent concentrations required to produce half-maximum platelet aggregations during poor glycaemic state were calculated and compared with the concentrations needed to yield the same aggregation amplitudes after the recovery. **Results**: Median time for the achievement of glycaemic control was 58 days (range 45-69). Median fasting plasma glucose levels decreased significantly from 205 mg/dl to 121 mg/dl ($p=0.003$). Median HbA_{1c} levels reduced significantly from 9.6% to 6.6% ($p=0.003$) indicating to restoration of glycaemic control. A small but significant decrease was observed in median total cholesterol levels (205 mg/dl vs. 192 mg/dl at poor and good metabolic states, respectively, $p=0.045$). Median triglyceride and fibrinogen levels did not change significantly with the restoration of glycaemic control. Median ADP and collagen concentrations required to produce half-maximum platelet aggregation amplitude at the initial evaluation was significantly lower than the value at the final evaluation (ADP: 1.79 μM vs. 3.17 μM at poor and good glycaemic control, respectively, $p=0.02$; collagen: 0.7 mg/ml vs. 1.01 mg/ml at poor and good glycaemic control periods, respectively, $p=0.006$) **Conclusions**: The data clearly indicate that platelet aggregability significantly decreases in type 2 diabetic patients after the restoration of glycaemic control.

PS 110
Nitric Oxide

1222

ASSESSMENT OF PLASMA NITRIC OXIDE CONCENTRATION IN TYPE 1 DIABETIC PATIENTS WITH MICROANGIOPATHY

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Nitric oxide (NO) is a short-lived messenger molecule serving as a transmitter in different tissues and is metabolized to NO_2^- and NO_3^- in humans. NO plays an important role in pathogenesis of Type I diabetes and probably it's late complications. **Aims:** the aim of the study was to estimate plasma nitrite anion (NO_2^-) concentration in Type I diabetic patients with and without late diabetic complications. **Materials and methods:** the study was performed in 20 patients with diabetic retinopathy and/or nephropathy (11 female and 9 male, aged 30.3±10.6 years, duration of diabetes 13.5±4.6 years, HbA1c 8.3±2.1 %) (group A) and 25 patients without diabetic complications (15 female and 10 male, aged 28.3±9.0 years, duration of diabetes 5.4±1.8 years, HbA1c 7.8±3.2 %) (group B). NO_2^- concentration was measured with the use of a colorimetric micromethod, where nitrate reductase catalyses the conversion of NO_3^- to NO_2^-. **Results:** the NO_2^- plasma concentration was significantly higher in diabetic patients in comparison with controls. We did not observe any differences between NO_2^- plasma concentration in the two groups of diabetic patients (group A, group B, healthy subjects: 40.24±4.27, 36.87±3.49, 16.68±2.16 µmol/l, respectively, $p>0.05$, $p<0.05$, $p<0.0001$). The nitrite concentrations did not correlate with HbA1c ($r=-0.03$, $p>0.05$). **Conclusions:** the results support the concept that metabolism of nitric oxide in diabetes is disturbed independently from the presence of late diabetic complications.

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PROSTAGLANDIN-MEDIATED VASODILATORY ACTION IN RESPONSE TO ACETYLCHOLINE INFUSION IS GREATER IN PATIENTS WITH TYPE 1 DIABETES COMPARED WITH CONTROLS

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Aims To determine whether conflicting reports of impaired endothelial function in type 1 diabetes, as measured by the vasodilatory response to infused acetylcholine [ACh] are due in part to methodological differences affecting the availability of vasodilatory prostanoids.

Materials and Methods We examined the role of endogenous prostanoids in twelve healthy, normotensive type 1 diabetic adults (6M 6F, age 34.5 ± 1.9 yr [mean±SEM], duration of diabetes 13.4 ± 1.9yr) and twelve controls matched for age, sex and BMI. Forearm blood flow [FBF] was measured using a venous occlusion plethysmography technique. Vasodilatory responses were measured at baseline and following brachial artery infusions of ACh (7.5, 15, 30 µg/min). Subjects' vasodilator responses at baseline and after ACh were then re-examined following local intra-arterial infusion of indomethacin [IND] (0.3mg/100ml forearm volume over 20 min), a cyclo-oxygenase inhibitor. Absolute values of FBF were examined.

Results Following IND, the vasodilator responses to all ACh doses were reduced in both groups. However, at higher doses of ACh (15 & 30µg/min) the reduction in blood flow following IND was greater in diabetic patients compared with controls.

	Reduction of FBF (%) in type 1 diabetes	Reduction of FBF (%) in controls	p-value
Baseline	3.47 ± 0.49	4.15 ± 0.56	0.19
ACh 7.5 mcg/min	22.84 ± 5.18	19.14 ± 5.49	0.31
ACh 15 mcg/min	25.77 ± 5.14	11.93 ± 5.46	<0.04
ACh 30 mcg/min	23.45 ± 4.60	10.27 ± 2.05	<0.04

Conclusions Vasodilatory prostanoids are important in determining endothelial response to ACh in diabetic and non-diabetic subjects. In addition, increased prostaglandin-mediated vasodilation may be compensating for attenuated responses to nitric oxide in diabetic subjects. These findings may partly explain the conflicting reports of endothelial dysfunction in patients with type 1 diabetes.

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EFFECT OF HYPERGLYCAEMIA ON SYSTEMIC AND RENAL PRODUCTION OF NITRIC OXIDE METABOLITES AND cGMP EXCRETION IN IDDM PATIENTS

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Nitric oxide (NO) plays an important role in regulation of systemic and regional haemodynamics via activation of soluble guanylate cyclase. The increase in cyclic guanosine 3´5´monophosphate (cGMP) is accompanied by a vascular relaxation. **Aims:** The aim of our study was to evaluate the effect of acutely-induced hyperglycaemia on systemic and renal NO production and urinary cGMP excretion. **Methods:** We measured the serum and urinary nitrite/nitrate (NO_x) concentrations and urinary cGMP excretion in two 90-minute periods under glycaemic clamp-induced euglycaemia (period I, 5 mmol/L) and hyperglycaemia (period II, 12 mmol/L) in a group of 20 patients with insulin-dependent diabetes mellitus (IDDM) without microalbuminuria and 15 weight-, age- and sex-matched healthy controls (C). **Results:** During period I NO_x excretion was comparable in IDDM and C and significantly increased during hyperglycaemia in C (345 ± 241 vs 454 ± 278 nmol.min^{-1}, $p < 0.01$), while it did not change in diabetics (355 ± 239 vs 326 ± 166 nmol.min^{-1}). No significant differences in urinary cGMP excretion (IDDM: 1139 ± 627 vs 1051 ± 1002 pmol.min^{-1}; C: 1212 ± 693 vs 1216 ± 731 pmol.min^{-1}) and serum NO_x (IDDM: 19 ± 9 vs 18 ± 9 umol.L^{-1}; C: 22 ± 12 vs 21 ± 10 umol.L^{-1}) were found between periods I and II in both groups. There were no significant relationship between changes in urinary NO_x and cGMP. **Conclusions:** The IDDM patients have an impaired regulation of NO production in kidneys with possible impact on sodium homeostasis and renal haemodynamics. (Supported by grant IGA MZ ČR, No 4242-3)

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GLUCOSE-MEDIATED FUNCTIONAL ALTERATIONS IN RESISTANCE ARTERIES ISOLATED FROM PATIENTS WITH IDDM.

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Introduction: Hyperglycaemia is associated with the development of diabetic microangiopathy but the exact nature and causes of the abnormalities that develop in the resistance vasculature are unclear. **Aims:** To determine whether raised glucose levels preferentially alter the function of endothelial or smooth muscle cells in arteries from patients with insulin-dependent diabetes mellitus (IDDM). **Methods:** Resistance arteries were isolated from gluteal subcutaneous fat biopsies from 8 patients with IDDM and 6 non-diabetic controls. Following 1hr incubation in physiological salt solution (PSS) containing either normal (5.5mM) or elevated glucose (20mM) concentrations, contractile responses were obtained for receptor-dependent (noradrenaline (NA), endothelin-1 (ET-1)) and receptor-independent (potassium solution (K^+)) vasoconstrictors. Relaxation was assessed using the endothelium-dependent, receptor-dependent vasodilators acetylcholine (ACh) and bradykinin (BK) and the endothelium-independent, exogenous nitric oxide (NO) donor, 3-morpholinosydnonimine (SIN-1). **Results**: Vasoconstrictor responses to NA, ET-1 and K^+, and vasodilator responses to ACh and BK were similar in arteries from patients and controls and were unaltered by high glucose concentrations. The sensitivity (-logIC_{50}), but not the size, of the relaxation with exogenous NO (SIN-1) was slightly, but not significantly (P=0.18), enhanced in arteries from patients with IDDM (6.81±0.17) compared with controls (6.46±0.14). Exposure to raised glucose caused a rightward shift (desensitisation) of this response which was significant (P=0.03) in arteries from IDDM patients (6.32+0.11) but not in those from controls (6.28±0.14; P=0.46). **Conclusions:** This study confirms our previous observation that functional responses are unaltered in resistance arteries from patients with IDDM and demonstrates that these responses are largely unaffected by an acute elevation of glucose concentrations *in vitro*. The selective, glucose-mediated reduction in sensitivity to exogenous NO in resistance arteries from patients with IDDM suggests an alteration in the NO pathway which may develop as an adaptive response to hyperglycaemia *in vivo*. This may contribute to the development of vascular complications in IDDM.

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MUSCLE VASODILATATION BY C-PEPTIDE IS NO-MEDIATED

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Recent studies suggest that C-peptide increases blood flow in both exercising and resting forearm in patients with IDDM.

Aims: To find out whether the C-peptide effect on forearm muscle blood flow is NO-mediated.

Material and methods: Eleven IDDM patients, aged 37±4 yrs with a diabetes duration of 23±4 yrs, were studied twice within one month. Their mean HbA1c was 7.2±0.7%. On both study occasions they received i.v. insulin infusion so regulated that euglycemia (5-6mmol/l) was achieved during the study. Forearm blood flow was measured by venous occlusion plethysmography during both arterial and i.v. infusions of saline or C-peptide (12 nmol/2 min i.a., and 6 pmol/kg/min i.v.) as well as during i.a. infusions of acetylcholin (Ach, 20µg/2 min) and sodium nitroprusside (Np, 20µg/2min) before, during or after i.a. infusion of a NO synthase inhibitor (L-NMMA, 28 mg/24min).

Results: I.v. infusion of C-peptide increased basal forearm blood flow by 34 ± 10%, $p<0.01$, whereas the blood flow was unchanged during saline infusion (-5.0 ± 3%). I.a. administration of L-NMMA reduced blood flow by 41 ± 4% during i.v. infusion of C-peptide and by 26 ± 7 % during saline infusion. The reduction in blood flow was more marked during C-peptide infusion ($p<0.05$) than during saline. Forearm blood flow did not change when C-peptide (12 nmol/2min) or saline were combined with the i.a. administration of L-NMMA. However, when the same dose of C-peptide was repeated i.a. 60 min later, forearm blood flow increased by 30% ($p<0.001$). I.a. infusions of Ach and Np raised forearm blood flow by approx 200 and 400%, respectively, both during i.v. infusion of C-peptide and saline. L-NMMA blocked the acetylcholine response by nearly 80% (from 200 to 45%) both during intravenous saline and C-peptide infusions periods, whereas the response to Np was unchanged.

Conclusion: The vasodilatory effect of C-peptide in skeletal muscle in IDDM patients is NO-mediated

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RESPONSE TO PROLONGED SYSTEMIC NO INHIBITION IN TYPE 2 DIABETIC PATIENTS AND CONTROLS. EFFECTS OF NIFEDIPINE.

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Abnormalities of endothelial function leading to impaired NO release and vasoconstriction have been postulated in Type 2 diabetic patients (D2) with (M+) or without microalbuminuria (M-). Prolonged systemic infusion of N^{G}-monomethyl-L-arginine (L-NMMA) can simulate chronic, generalized impairment of NO release from endothelium in vivo. One can suggest that systemic response to L-NMMA is reduced in D2M+ and/or in D2M- because of lower baseline NO release. Aims: to study the effects of prolonged infusion of L-NMMA (540 mg/2hours) alone and in association with sublingual route nifedipine administration (20 mg). Methods: We evaluated cardiac index by ultrasonography (CI: ml/min/1.73m2); mean blood pressure (MBP: mmHg); renal plasma flow (RPF: ml/min/1.73m2); glomerular filtration rate (GFR: ml/min/1.73m2) and renal vascular resistances (RVR mmHg/ml/min/1.73m2) in 5 D2 M+, in 5 D2 M- and in 5 controls (C), all males and with mild arterial hypertension. Results: L-NMMA decreased baseline CI (mean ± S.E.) (C vs D2M- vs D2M+: 2699±88 vs 3257±353 vs 2909±147) by 11±3% in C, 16±5% in D2M- and 6±3% in D2M+ (n.s.). A 7±3% MBP rise was observed in C, 5±4% in D2M- and 12±1% in D2M+ from baseline (C vs D2M- vs D2M+: 114±10 vs 115±5 vs 113±3) (n.s.); a 19±3% RPF decrease was observed in C, 12±2% in D2M- and 12±4% in D2M+ from baseline (C vs D2M- vs D2M+: 440±43 vs 662±120 vs 403±49) (n.s.). A 7±1% GFR decrease was observed in C, 9±3% in D2M- and 6±3% in D2M+ from baseline (C vs D2M- vs D2M+: 89±4 vs 102±3 vs 98±5) (n.s.). A 33±4% RVR increase was observed in C, 16±4% in D2M- and 26±8% in D2M+ from baseline (C vs D2M- vs D2M+: 0.15±0.03 vs 0.17±0.02 vs 0.11±0.02) (n.s.). Nifedipine completely reversed L-NMMA induced MBP increase, restored CI and attenuated renal vasoconstriction. Conclusions:1) sustained NO inhibition is accompained by systemic and renal vasoconstriction both in C, D2M+ and D2M- without significant differences in the 3 groups; 2) Nifedipine reverses cardiovascular and renal effects induced by sustained systemic NO deficiency both in C and D2.

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THE EFFECT OF 5-AMINOIMIDAZOLE-4 CARBOXAMIDE RIBOSIDE (AICA-r) ON THE RESPONSES OF ISOLATED THORACIC AORTA IN STREPTOZOTOCIN DIABETIC RATS

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Aim : AICA-r, the nucleoside corresponding to AICA- ribotide, an intermediate of the de novo pathway of purine biosynthesis was recently proposed as a new insulinotropic tool in non- insulin- dependent diabetes mellitus. The major aim of the present study was to define whether AICA riboside affects the vascular responsiveness to vasoconstrictors and vasodilators in thoracic aorta of streptozotocin (STZ)- diabetic rats. **Materials and Methods :** Studies were performed in untreated control rats (n=11), neonatal- STZ- induced (100 mg/kg, i.p. at the second day of their birth and were then maintained 16 weeks) diabetic rats (n=5), AICA- treated (10 mg/kg/day, i.p. for one month, beginning at the 16th week) control rats (n=5) and AICA- treated (10 mg/kg/day, i.p. for one month, beginning at the 16th week) diabetic rats (n=7). Two thoracic aorta rings were taken from all animals and prepared two subgroups (endothelium intact, E+, and denuted, E-, strips).Results are expressed as the mean±s.e.mean. Relaxant responses are expressed as a percentage (%) relaxation of noradrenaline- induced tone. Statistical analysis of the data was performed using one- way analysis of variance (ANOVA) followed by Tukey- Kramer multiple comparisons test. $P<0.05$ was considered as statistically significant. **Results :** One month AICA-r treatment : 1. significantly increased the body weights of diabetic rats (181±13 to 239±2 g, $p<0.001$) 2. significantly decreaesd the blood glucose levels of diabetic rats (302±47 to 135±11 mg/dl, $p<0.001$) 3. did not significantly affect fast, slow and total components of responses to noradrenaline in all the experimental groups 4. reversed the increased max. contraction values of noradrenaline in diabetic rats to near- control values (E+: 300±5 to 204±19 mg tension/mg ww, $p<0.05$) 5. reversed the completely abolished responses of acetylcholine (pD2 and % relaxation) in diabetic rats to control values 6. reversed the decreased pD2 values of sodium nitroprussiate in diabetic rats to control values. **In conclusion :** AICA-r treatment could improve the increased blood glucose level, accelerate gaining weight, reverse the endothelial dysfunction and normalize the vascular responses in neo- STZ- diabetic rats.

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IMPAIRED RELAXATION IN AORTA FROM STREPTOZOTOCIN-DIABETIC RATS : EFFECT OF AMINOGUANIDINE TREATMENT.

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Aim : The effect of 8 weeks' streptozotocin (STZ)- induced diabetes and aminoguanidine (AG), the inhibitor of advenced glycosylation reaction, treatment on arteriolar reactivity to vasoactive substances was investigated in vitro. **Materials and Methods :** Studies were performed in untreated control rats (n=10), STZ- induced (60 mg/kg i.v.) diabetic rats (n=10), AG- treated (600 mg/l given in drinking water throughout 8 weeks) control rats (n=10) and AG- treated (600 mg/l given in drinking water, beginning at 72 h after streptozotocin and throughout 8 weeks of diabetes) diabetic rats (n=10). Resulrs are expressed as the mean± s.e.mean. Relaxant responses are expressed as a percentage (%) relaxation of noradrenaline- induced tone. Statistical comparisons were made by one- way analysis of variance (ANOVA) followed by Tukey- Kramer multiple comparisons test. $P<0.05$ was considered as statistically significant. **Results :** 1. The decreased body weights (205±6 g) and increased blood glucose levels (583±8 mg/dl) of diabetic rats were partially restored by treatment of aminoguanidine (253±6 g, $p<0.05$ and 480±14 mg/dl, $p<0.001$, respectively). 2. Diabetes caused a 71% deficit in maximal endothelium- dependent relaxation (23±5%) to acetylcholine for noradrenaline precontracted aortas ($p<0.001$). Aminoguanidine treatment prevented the diabetes- induced impairment in endothelium dependent relaxation (58± 8%) to acetylcholine, maximum relaxation remaining in the non- diabetic range (78±4%). 3. Neither diabetes nor treatment affected endothelium- independent relaxation (pD2 and max. İnhibition) to sodium nitroprussiate. 4. Vasoconstrictor responses (pD2 and max. Contraction) to noradrenaline and KCl were not influenced by the the diabetic state and treatment. **In conclusion :** These data suggest that 8 weeks of experimental diabetes is associated with a decreased endothelium- dependent vasodilatation. AG treatment may prevent diabetes- induced endothelial dysfunction. This may be mediated via the prevention of advanced glycosylation end product formation, the enhanced release of vasodilator substances such as prostacyclin, the increased elasticity of blood vessels, the antioxidant activity and inhibitor activity of enzyme aldose- reductase of aminoguanidine.

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CORRELATIONS BETWEEN MORPHOLOGICAL AND FUNCTIONAL MICROCIRCULATORY ALTERATIONS IN DIABETES AND THE IMPACT OF METABOLIC CONTROL AND DISEASE DURATION

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Only minor changes of skin capillary morphology have been described in diabetes by means of capillaroscopy without dyes. **Aims:** To examine correlations between functional and morphological abnormalities of the capillaries in type 1 and 2 diabetes and the influence of metabolic control and diabetes duration on capillary morphology. **Materials and Methods:** Sixteen type 1 and 19 type 2 diabetic patients were investigated and two age- and sex-matched groups comprising 16 and 19 non-diabetic subjects served as controls. Capillary density and morphology was evaluated at the distal row of nailfold capillaries. Capillary blood cell velocity (CBV) was measured in the dorsal middle phalangeal area during rest and after 3-min arterial occlusion by means of laser Doppler anemometry. **Results:** Capillary density, width and arterial limb diameter were similar in type 1 and 2 diabetic patients compared to their controls. Capillary diameters of the apical part and the venous limb were slightly enlarged both in type 1 and 2 diabetes, but a significant difference only was found when type 1 and 2 diabetic patients were compared in common to their controls (apex: 19.2±0.6 µm vs. 17.4±0.6 µm, $p<0.05$; venous limb: 17.3±0.5 µm vs. 15.9±0.4 µm, $p<0.05$). Tortuos capillaries were more often observed in type 1 (n=13 vs. n=7, $p<0.05$) and 2 diabetic patients (n=16 vs. n=9, $p<0.05$) than in controls. In type 1 diabetes an inverse correlation (r=-0.52; $p<0.05$) was noticed between capillary density and resting CBV. In type 2 diabetic patients a positive correlation (r=0.49; $p<0.05$) was found between the capillary apex diameter and peak CBV. No association between metabolic control, evaluated by HbA_{1c} levels, and capillary morphology was found, whereas disease duration was inverse correlated to arterial limb diameter (r=-0.48; $p<0.05$) and width of the capillaries (r=-0.48; $p<0.05$) in type 2 diabetes. **Conclusions:** In type 1 and 2 diabetic patients only minor, unspecific changes of capillary morphology were found. Slight correlations between morphological and functional alterations may be explained by haemodynamic rather than metabolic factors.

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VASCULAR FUNCTION AND AGEING IN SUBJECTS WITH A GENETIC PREDISPOSITION TO TYPE 2 DIABETES

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Diabetes is a known risk factor for cardiovascular disease and has often been likened to accelerated ageing of the vasculature. Normoglycaemic offspring of Type 2 diabetic parents have previously been shown to have metabolic abnormalities and are at increased genetic risk of developing of diabetes. It is unclear whether genetic predisposition might be itself be associated with accelerated ageing effects on vascular function. **Aims:** To explore the relationship vascular function and ageing in this at risk population. **Methods:** Using laser Doppler fluximetry we assessed maximum microvascular hyperaemia (MMH) to local heating of the skin in 21 non-obese, glucose-tolerant offspring of two Type 2 diabetic parents (group 2) and 21 age and sex matched controls (group 1), (10 M, age 40 [19-54] vs 42 [21-55]yrs, median and range, $p=0.39$). All underwent OGTT and serum insulin and plasma glucose were measured. **Results**: MMH did not differ between the two groups, However minimum microvascular resistance (mean BP/MMH) was correlated with age in group 2 but not in group 1, ($Rs=0.47$, $p=0.03$, Spearman rank). Fasting plasma glucose was similar in the two groups but the two hour values and the area under glucose curve were significantly higher in group 2 compared to group 1, (6.4 [5.3-6.6] vs 4.9 [4.2-4.6] mmol/l, $p=0.005$) and (13.6 [10.8-17.4] vs 12.0 [9.1-15.1] mmol/l h, $p=0.001$ Mann-Whitney) respectively. **Conclusions:** In individuals predisposed to Type 2 diabetes, with subtle derangements of glucose metabolism, the effects of age on vascular function appears to be more pronounced than in matched controls.

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ENDOTHELIAL DYSFUNCTION AND INSULIN SENSITIVITY: A RELATIONSHIP MODULATED BY THE LIPID PROFILE.

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Aims: Endothelial behavior is altered in patients with established atherosclerotic disease and in those with risk factors related to insulin resistance (IR) but without overt atherosclerosis. Several data suggest that endothelial dysfunction and IR are linked. Hypercholesterolemia is associated with impaired endothelial function, but not with IR. **Materials and Methods:** The link between endothelial dysfunction (transcapillary leakage of albumin, TERalb) and IR (HOMA) has been studied in 127 non-diabetics: 23 healthy controls (C), 47 hypertensives with no overt atherosclerosis (EH), 12 subjects with familial hypercholesterolemia (FH), 30 normotensives with overt atherosclerosis (ATH) and 15 subjects with hypertension and atherosclerosis (EH/ATH). **Results:** EH and FH were younger, BMI was higher in EH and EH/ATH. ATH, EH/ATH and FH had higher LDL-ch ($p<0.0001$) and ApoB ($p=0.004$). LDL/ApoB ratio was higher in FH ($p<0.01$) and similar in the other four groups. ATH and EH/ATH had higher triglycerides ($p=0.02$). 24 hour sBP and dBP were higher ($p<0.001$) in EH and EH/ATH, and similar in C, FH and ATH. TERalb was 6.5±1.6 %/h in C, higher (logTERalb, ANOVA, $p=0.0001$) in EH (9.1±2.6 %/h, $p<0.01$), ATH (9.9±2.4, $p<0.001$) and EH/ATH (10.8±3.3, $p<0.001$), and intermediate in FH (7.9±1.1%). HOMA IR was similar in C: 2.22±1.11; EH: 2.59±1.32; FH: 2.11±0.75; ATH 2.27±1.38 and EH/ATH: 2.57±1.03. A positive regression was found between TERalb and HOMA IR (n. 127, r=0.30, $p=0.0015$) and remained after controlling for age, BMI, smoking, LDL and triglycerides and presence of atherosclerosis or hypertension. After stratifying by LDL/ApoB quartiles, such regression drops in the 1st (r=0.57, $p=0.79$) and 2nd quartile (r=0.19, $p=0.36$), while persists in the 3rd (r=0.46, $p=0.015$) and 4th quartile (r=0.40, $p=0.03$). **Conclusions:** Our data suggest that insulin sensitivity (HOMA IR) is a significant "predictor" of endothelial dysfunction (widespread permeability) in those patients with high LDL/ApoB ratio.

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MICROVASCULAR FUNCTION AND FEATURES OF INSULIN RESISTANCE SYNDROME IN HEALTHY ADULTS

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Microvascular complications are often present when diabetes is diagnosed later in life. This may be due to a long period of undiagnosed diabetes or may indicate that abnormalities of microvascular function precede diabetes. Previous studies have demonstrated that cutaneous microvascular vasodilatory capacity is decreased in subjects with fasting hyperglycaemia who have only mild derangements of glucose metabolism but no diabetes and in this group abnormalities are related to insulin sensitivity. Whether such abnormalities predate fasting hyperglycaemia or are influenced by the subjects' insulin resistance are unknown. **Aims:** (1) To investigate whether microvascular function is deranged in normoglycaemic subjects at high genetic risk of Type 2 diabetes and (2) To explore the links between microvascular function and an individual's sensitivity to the actions on insulin. **Methods:** Using laser Doppler fluximetry we assessed maximum microvascular hyperaemia (MMH) to local heating of the skin in 21 non-obese, glucose-tolerant offspring of two Type 2 diabetic parents (10 M, age 40 [19-54] yrs, median and range), and 21 age and sex matched controls. Blood was taken for PAI-1, vWF, tPA, lipids, fasting glucose and insulin. **Results**: MMH did not differ between the two groups, (1.53 [0.84-2.55] V vs 1.56 [0.86-2.19] V controls, Mann-Whitney). Significant correlations (Spearman) between minimum microvascular resistance (mean BP/MMH) and insulin resistance were observed in the combined group (Triglyceride $p=0.024$, Total cholesterol $p=0.022$, PAI-1 $p=0.003$, tPA, $p=0.026$) and an inverse correlation with insulin sensitivity ($p=0.031$). **Conclusions:** The MMH was not abnormal in subjects at increased risk of diabetes. However the data provide evidence to support the association between features of the insulin resistance syndrome and microvascular dysfunction.

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IMPROVEMENT IN ENDOTHELIAL FUNCTION DEPENDS MAINLY ON BLOOD PRESSURE BUT NOT GLUCOSE CONTROL IN TYPE 2 DIABETES

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Aims: Type 2 diabetic patients suffer from endothelial dysfunction that can be demonstrated by a reduced flow associated vasodilatation (FAD%) using high resolution ultrasound. We were interested in the question whether amelioration of glucose metabolism would restore endothelial function.

Patients and Methods: We studied 24 consecutive type 2 diabetic patients from our outpatient clinic (11 f, 13 m, age 52.3±15.2 y, BMI 32.8±7.5 kg/m^2, diabetes duration 8.1±9.1 y) with insufficient diabetes control. FAD% was measured at first visit and after intensifying antidiabetic treatment 3 months later. A high resolution ultrasound system with a transducer up to 13.0 MHz and an axial resolution of 0.12 mm was used for the determination of FAD%. Values are given as means ± SD, significance was tested by paired samples T-test and correlation by ANOVA.

Results: HbA1c improved from 9.3±1.8 % to 7.6±1.5% (delta HbA1c = 1.7%, P=0.0008), but was not related to an improvement in FAD% (4.13±2.8% to 4.6±2.9%, delta FAD% = 0.47 %, P = 0.67). Although mean total cholesterol, triglycerides, and blood pressure were not influenced significantly, there was a weak but not significant negative correlation between delta FAD% and the delta total cholesterol (R = -0.27, P = 0.21). In those patients that changed blood pressure there was a clear negative correlation between delta systolic blood pressure [-50 to +30 mmHg] and delta FAD% [- 7.5 to +14%](R = -0.47, P = 0.02). Conclusions: Our data indicate that improvements in endothelial function in type 2 diabetic patients are not likely to be caused by an improvement in glucose control alone, whereas lowering of the systolic blood pressure will probably have the most favourable effect on endothelial function in these patients. This underlines the need for a multifactorial approach in the management of diabetes and its complications.

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DIETARY FATTY ACIDS INFLUENCE BOTH INSULIN SENSITIVITY AND ENDOTHELIUM DEPENDENT VASOREACTIVITY IN TYPE 2 DIABETES.

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Insulin resistance and impaired endothelial-dependent vasoreactivity predict atherosclerosis. **Aims** to examine the effect of an oleic acid-rich diet on insulin resistance and endothelium dependent vasoreactivity . **Materials and Methods** 11 type 2 diabetic patients were changed from the usual linoleic acid-rich diet to an oleic acid-rich diet for 2 month. Insulin-mediated glucose transport was measured in isolated adipocytes. Fatty acid composition of the adipocyte membranes was determined by gas-liquid chromatography. Endothelium dependent and independent flow mediated vasodilatation (FMD) were measured in the superficial femoral artery. **Results** There was an increase in oleic acid and decrease in linoleic acid on the oleic acid-rich diet ($p<0.0001$). Diabetic control was unchanged but fasting glucose/insulin decreased on the oleic acid-rich diet ($p<0.05$). Insulin-stimulated glucose transport was greater on the oleic acid-rich diet (0.56±0.17 vs 0.29±0.14 nmol/10^5 cells/3mins, $p<0.0001$). Endothelium dependent FMD was greater on the oleic acid-rich diet (3.90±0.97% vs 6.12±1.36% $p<0.0001$). There was a significant correlation between adipocyte membrane oleic/linoleic acid and insulin mediated glucose transport ($p<0.001$). There was a significant positive correlation between adipocyte membrane oleic/linoleic and endothelium dependent FMD ($r=0.61, p<0.001$) but no relationship between insulin stimulated glucose transport and change in endothelium dependent FMD. **Conclusions** A change from polyunsaturated to monounsaturated diet in type 2 diabetes reduced insulin resistance and restored endothelium dependent vasodilatation suggesting an explanation for the anti-atherogenic benefits of a Mediterranean-type diet.

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ACE GENE POLYMORPHISM IS A POTENTIAL RISK FACTOR NOT FOR MACROANGIOPATHY,
BUT FOR MICROANGIOPATHY IN JAPANESE NIDDM.

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Back Ground:ACE polymorphism has been reported to be a candidate risk factor for the development and progression of diabetic nephropathy. Recently, we reported the significant association between ACE polymorphism and diabetic retinopathy as well as nephropathy in Japanese NIDDM.

Aim: This study was initiated to analyze the relationship between macroangiopathy and ACE gene polymorphism in Japanese NIDDM.

Materials and Methods: Japanese NIDDM patients who had been diabetic longer than 10 years (N=111, male: N=58, female: N=53, total average age: 60years) were recruited to this study. ACE gene polymorphism was determined by PCR methods using DNA from patients' peripheral white blood cells. We followed for 7 years to see whether these patients had macroangiopathy, such as angina pectoris, myocardial infarction, cerebral vascular attack and ASO. Nephropathy was determined by the urinary albumin index$\geqq$10 mg/g.creatinine. Retinopathy was diagnosed by two independent opthalomologists.

Results: Among 111 patients, eight patients had cardiovascular events (DD: N=1, ID: N=2, II: N=5), and eleven patients had cerebral vascular attacks(ID: N=5, II: N=6). There was no patient with ASO. In this study, we did not find a significant association between ACE gene polymorphism and macroangiopathy. In contrast, the D-allele of ACE gene was significantly more frequent in the patients with nephropathy($P=0.036$, by X^2 test) and Retinopathy($P=0.047$, by X^2 test) in the same patients above.

Conclusion: Genetic risk factor for macroangiopathy is different from that for microangiopathy.

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LIPOPROTEINLIPASE MODULATES GLYCATED LDL INDUCED EFFECTS IN CULTURED VASCULAR ENDOTHELIAL CELLS.

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Hyperglycemia is closely related to the occurrence of late diabetic vascular complications. Glycated lipoproteins are suggested to be involved in these processes. Aims: This study was thus designed to evaluate the effects of glycated LDL (low density lipoprotein) in HUVECs (human umbilical vein endothelial cells). Methods: Proliferation, apoptosis and protein expression were measured by 3H-thymidine assays and Western blot analysis. In vitro glycated LDL as well as glycatéd and non glycated LDL fractions from diabetic patients were used for the experiments. Results: HUVECs, incubated (24 - 48h) with in vitro glycated LDL (100 µg/ml), exhibited reduced proliferation (70%), but increased apoptosis (120%) compared to their intraindividual control cells cultivated with non glycated LDL. In parallel expression of endothelial NO-Synthase (eNOS: 130%), Retinoblastoma protein (pRb: 131%) and of p27 (130%) were increased. Addition of lipoproteinlipase (LPL- 0.1 U/ml) did not affect proliferation and Cyclin D3-expression, but modulated basal as well as glycated LDL induced apoptosis and reduced eNOS (65%), pRb (71%) and p27 (63%). Exposure of HUVECs to glycated LDL from diabetic patients slighty reduced proliferation (91%) versus their intraindividual non glycated LDL fraction, associated with a diminished Cyclin D3 expression (87%). Increased rates of apoptosis were exclusively observed in the presence of LPL (130%).In none of the LDL preparations oxidation products were detectable. Conclusions: The observations, that in vitro and in vivo glycated LDL as well as LPL differentially modulate proliferation, apoptosis and associated protein expression in vascular endothelial cells suggest glycated LDL and LPL to have a potential role in the development of late diabetic vascular complications.

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Oxidative Stress

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HYPERGLYCEMIA DECREASES THE GLUTATHIONE CONTENT IN THE AORTIC TISSUE OF DIABETIC RATS

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Aims: Glutathione (GSH) redox cycle plays a major role in scavenging hydrogen peroxide (H_2O_2) under physiological conditions. Recently, we demonstrated that high glucose reduced H_2O_2 scavenging activity in human vascular smooth muscle cells. We also showed that high glucose decreased the intracellular GSH content and the rate of uptake of cystine, a rate-limiting factor in maintaining GSH levels (FEBS Lett.421:19-22,1998). We investigated here, whether hyperglycemia impairs the GSH redox cycle function in diabetic rats in vivo. Materials and Methods: Wistar rats were divided into three groups, streptozotocin-induced diabetic (STZ-D,n=7), insulin-treated STZ-D (I-STZ-D,n=8) and non-diabetic controls (C,n=7). The GSH content in extracted aortic tissue was enzymatically measured and expressed as n mol/mg protein. Furthermore, Otsuka Long-Evans Tokushima Fatty (OLETF) rats developed diabetes spontaneously and the correlation between the GSH content in the aortic tissue and blood glucose level was investigated. Results: The GSH content in the aortic tissue was significantly reduced in the STZ-D group (0.99±0.14 n mol/mg protein) compared with the C group (1.68±0.15 n mol/mg protein). Insulin treatment significantly restored the GSH content in the I-STZ-D group (1.45±0.11 n mol/mg protein). The GSH and blood glucose levels in all Wistar rats were significantly correlated (r = - 0.69, p<0.01, n=22). An identical correlation between GSH content and blood glucose was obtained in OLETF rats (r = - 0.64, p<0.05, n=10). Conclusions: Hyperglycemia causes impairment of the function of the GSH redox cycle in both STZ-induced diabetic Wistar rat and OLETF rat aortic tissue in vivo. Our results suggest that glycemic control prevents the development of diabetic macrovascular disorders, partly by maintaining the GSH redox cycle function.

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I/D ANGIOTENSIN CONVERTING-ENZYME GENE POLYMORPHISM IN IDDM PATIENTS: RELATIONSHIPS WITH AER AND OXIDATIVE STRESS.

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Aim: Oxidative stress seems to contribute to development of endothelial dysfunction and to diabetic complications. We studied the relationships between ACE gene polymorphism and endothelial dysfunction, measured by AER, and oxidative stress, evaluated by conjugated dienes(CD). Materials and Methods we studied 43 normotensive, normoalbuminuric Type 1 diabetic patients (mean age 33±10 yrs, mean duration of disease 13.9±8.5, mean HbA1c 7.2±0.8). We divided the patients according to the I/D polimorphism (19DD, 18ID, 6II). Results: AER levels were significantly higher in DD compared to ID (5,8±5,3 vs 3,6±2,9µg/min; p=0.02) and to II genotype (5,8±5,3 vs 2,8±2,4µg/min, p=0,01). DD and ID genotype showed higher levels of total CD compared to II (0.0378±0.0031 and 0.0372±0.0035 vs 0.0332±0.0034 AU, p=0.012 and p=0.018, respectively). Significative higher mean HbA1c levels was also associated to the presence of D allele (DD vs II 7.4±0.7 vs 6.7±1.1%, p<0.05; ID vs II 7.2±0.8 vs 6.7±1.1%, p<0.05). No differences we found in lipid profile, age, duration of disease and smoking habit among the three groups. Conclusions: Our data showed that I/D ACE gene polimorphism could influence AER and HbA1c levels in normoalbuminuric normotensive type 1 diabetic patients. The finding that patients with D genotype had higher levels of AER, CD and HbA1c suggest that these patients seems to be more prone to the effects of hyperglicemia and to glucotoxicity. Hyperglicaemia may induce, via the production of oxigen radical species, higher levels of peroxidation products, such as CD, and consequently, an higher rate of endothelial dysfunction.

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ERYTHROCYTE ANTIOXIDANT STATUS OF TYPE 2 DIABETIC PATIENTS WITH AND WITHOUT COMPLICATIONS

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Oxidative stress plays a role in the pathogenesis of late complications of diabetes mellitus. In erythrocyte lysate of 81 type 2 diabetic patients (age:58.9±9.6 years; BMI:29.5±5.3 kg/m^2) and 29 healthy subjects (age:48.2±5.1 years; BMI:27,2±3.7 kg/m^2) reduced and oxidised glutathione (GSH, GSSG), glutathione peroxidase (GSH-PX), superoxide dismutase, glutathione reductase (GSH-R), catalase activity (CAT) and thiobarbituric acid reactive substances were determined. Diabetic patients were divided according to carbohydrate, lipid status and existence of diabetic late complications. No significant difference could be observed between the controls and the diabetic group with good metabolic status (HbA_{1C}<7.5%, n=28). Compared to the controls poor metabolic status (n=53) was associated with a decrease in GSH (7.48±2.2 *vs* 8.79±2.4 µM/gHgb, p<0.05), GSSG (0.88±0.48 *vs* 1.19±0.5 µM/gHgb, p<0.05) GSH-PX activity (6.07±1.6 *vs* 7.05±1.45 U/gHgb, p<0.05), and a higher CAT activity (1.52±0.3 *vs* 1.31±0.26 BU/ml haemolysate, p<0.05). Lower GSH-PX (6.07±1.6 *vs* 6.93±1.29 U/gHgb, p<0.05) and higher CAT activity (1.52±0.3 *vs* 1.32±0.27 BU/ml haemolysate, p<0.05) were observed comparing diabetic patients with poor and good metabolic status. Considering lipid status, hyperlipaemic controls (triglyceride>1.7mmol/l, cholesterol>5.2 mmol/l, n=21) showed reduced GSH (8.2±1.97 *vs* 10.34±2.85µM/gHgb, p<0.05) compared to the normolipidaemic controls (n=8). Hyperlipaemic diabetic patients (n=67) had a decreased GSH (7.51±1.89 *vs* 10.34±2.85 µM/gHgb, p<0.05), GSSG (0.91±0.49 *vs* 1.38±0.67 µM/gHgb, p<0.05), GSH-PX activity (6.31±1.55 *vs* 7.71±1.6U/gHgb, p<0.05) compared to the healthy group. GSH level was lower in diabetic patients with (DC, n=29) and without complications (DNC,n=52) compared to the healthy group (n=29) (7.59±2.32 *vs* 8.79±2.4µM/gHgb; 7.67±1.36 *vs* 8.79±2.4 µM/gHgb, p<0.05). CAT activity was higher in the DNC group compared to the controls (1.45±0.3 *vs* 1.31±0.26 BU/ml haemolysate, p<0.05). Comparing the DC and DNC groups no significant difference was found in the examined parameters. In our study, changes in the antioxidant parameters were associated with the actually impaired carbohydrate and lipid status rather than with manifest late complications of type 2 diabetic patients. This result suggests that the free radical reactions and their consequences that play a role in the pathogenesis of diabetic late complications are more likely to be detectable locally.

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Postprandial incrases of circulating methylglyoxal in type-II diabetic and non-diabetic subjects.

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Aim: Methylglyoxal (MG) is an early glycation product and is implicated in the genesis of diabetic vascular disease. In diabetic patients elevated levels of circulating MG are observed but a large overlap is found between MG levels in diabetic and in non-diabetic subjects and only week correlations between glycemic control and blood MG levels are reported. No information exists on possible postprandial alterations of MG in either type-II diabetic or non-diabetic subjects. **Methods:** Circulating levels of MG were determined in 6 type-II diabetics (m/f 3/3, age 57±11 years, HbA1c 9.2±0.8%,) and 6 non-diabetic controls (m/f 3/3, age 53±6 years) before and up to 4 hours after a standardized breakfast (520 kcal, 65 g carbohydrates). MG in blood was assayed by derivatization with 1,2-diamino-4,5-dimethoxybenzene and HPLC of the resulting product with fluorescence detection. **Results:** At baseline concentrations of 160±82 nmol/l MG in diabetic and 235±140 nmol/l in non diabetic subjects were measured. After the meal MG levels in blood increased in diabetic patients 3.6-fold to 433±227 nmol/l (p< 0.019 vs. baseline). In non-diabetic controls a 2.7-fold increase up to 492±233 nmol/l (p< 0.016 vs. baseline) was observed. Although the difference between diabetics and non-diabetic controls is statistically not significant, the increase observed in diabetics was about 135 % of that found in non-diabetics. **Conclusions:** Our data provide evidence that in the postprandial state circulating MG levels in blood increase in type-II diabetics as well as in healthy controls.

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DETECTION OF METHYLGLYOXAL ADDUCTS IN THE URINE OF DIABETIC PATIENTS

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Aims: Advanced glycation end products play an important role in the development of tissue damage in diabetes mellitus. The aim of the present study was the investigation of the excretion of different glycation end products in the urine of 98 diabetic patients (73 with normal and 25 with elevated serum creatinine).

Methods: Methylglyoxal, an intermediate product of the glycation, formed with L-arginine in an in vitro model two fluorescent peaks. Using the fluorescent characteristics of these (excitation/emission: A=320/400 nm and B=340/425 nm) and the generally accepted wavelength of the so called non-specific advanced glycation end product (C=370/440 nm), the excretion of these products was investigated in the urine of diabetic patients.

Results: These three particular glycation end products showed significant intercorrelations in the urine: A vs. B: R=0.80, A vs. C: R=0.53, B vs. C: R=0.83 ($p<0.001$). Concentrations of these glycation end products in the urine correlated negatively with the serum creatinine in the range between 120-240 µmol/l, R values were in the case of A:-0.80, B:-0.84 C:-0.88 ($p<0.001$).

Conclusions: Data presented here verify that L-arginine-methylglyoxal adducts can be detected in the urine of diabetic patients. One of these adducts (340/425 nm) showed a close correlation with the excretion of the non-enzymatic glycation end product measured at 370/440 nm. Elimination of these products by the urine is decreased in the stage of early renal insufficiency leading to retention of them in the circulation and probably this way causing progression of diabetic nephropathy and atherosclerosis.

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POSTPRANDIAL PHASE AND GENERATION OF OXIDATIVE STRESS IN DIABETES: EFFECT OF ANTIOXIDANT VITAMINS.

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Aims: it is known that oxidative stress is involved in the pathogenesis of diabetes and especially in cardiovascular complications. Recently it has been demonstrated that the postprandial phase is followed by the generation of oxidative stress in diabetic patients. It is also known that although the assumption of natural antioxidant substances with meals prevents cardiovascular complications, actually the administration of supplements of vitamins does not seem to exert any effect. Considering that the maximal production of oxidative stress is during the postprandial phase we evaluated if this phase is the most advantageous for administration of supplements of vitamins. **Materials and methods:** we studied 10 type 2 diabetic patients (age: 58±2,4 M±SD) during: 1)administration of 600 Kcal meal, 2) administration of vitamin C (1 g) + vitamin E (400 mg), 3) meal + vitamins, 4) meal + regularly long term taken vitamins (3 weeks). The blood samples were taken at 0- 60- 120-180 minutes and glycaemia, insulinaemia, MDA ($p=0.01$ by ANOVA for repeated measures), TRAP (total radical antioxidant parameter) were measured out in each one. **Results:** during the meal we could observe an increase of MDA ($p=0.01$ by ANOVA for repeated measures) and a reduction of TRAP ($p=0.01$). The assumption of vitamins alone was unable to exert any effect. The administration of vitamins during the meal turned out to be more effective than the long term administration in preventing the generation of oxidative stress ($p=0.01$). **Conclusions:** these data indicate that the administration of antioxidant substances during the meal is more effective in preventig the meal induced oxidative stress generation. Iit may be suggested that vitamins could reduce oxidative stress also blocking radical production within the bowel.

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IMPROVEMENT OF ENDOTHELIAL FUNCTION IN WOMEN WITH A HISTORY OF GESTATIONAL DIABETES MELLITUS (GDM) AFTER SHORT TERM ADMINISTRATION OF ASCORBIC ACID

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Aims: We have recently shown that both obese as well as non-obese normoglycemic prior-GDM women have disturbed endothelial function of conduit arteries associated with insulin resistance. As antioxidant therapy is known to improve impaired endothelial function in other disease states such as Diabetes Mellitus, we tested the hypothesis that acute oral administration of ascorbic acid might have a short term beneficial effect in this population. Materials and Methods: Seventeen women with a history of GDM, age 36.4±3.9yrs, BMI 28.2±6.6kg/m^2 and basal insulin resistance (HOMA) 3.8±2.7 were enrolled in a prospective randomised, double blind cross-over trial. High-resolution echo Doppler Ultrasound was used to measure right brachial artery changes during reactive hyperemia, (an endothelium dependent vasodilator - FMD) and after 0.4 mg of nitroglycerine sublingually (an endothelium independent vasodilator - NID). Each subject was assessed on two occasions: on day 1 baseline FMD was estimated and 2g ascorbic acid or placebo was given orally. Two hours later FMD and NID were estimated. On day two the same procedure was repeated with the opposite treatment. Results: Ascorbic acid administration resulted in a significant improvement of FMD (2.6±2.7% to 9.0±3.3%, $p<0.05$), whereas no improvement was observed after placebo (3.0±2.3% to 2.6±1.9%, ns). NID was similar after ascorbic acid or placebo. Conclusions: Oral administration of ascorbic acid acutely improves endothelium dependent vasodilatation in the brachial artery in prior-GDM women with various degrees of insulin resistance. This finding supports the hypothesis that insulin resistance *per se* may play a role in the increased oxidative stress which contributes to endothelial dysfunction.

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ANTIOXIDANT EFFECT OF 5- AMINOIMIDAZOLE- 4- CARBOXAMIDE RIBOSIDE (AICA-r) IN NEONATAL- STREPTOZOTOCIN- INDUCED DIABETIC RATS

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Aim : Free radical- mediated oxidative stress has been implicated in the development and exacerbation of several degenarative diseases such as diabetes mellitus. The present study was designed to investigate whether AICA-r has the antioxidant effect in some tissue of diabetic rats.

Materials and Methods : Studies were performed in untreated control rats (n=5), neonatal- streptozotocin (STZ)- induced (100 mg/kg, i.p. at the second day of their birth and were then maintained 16 weeks) diabetic rats (n=5), AICA- treated (10 mg/kg/day, i.p., for one month, beginning at the 16th week) control rats (n=5) and AICA- treated (10 mg/kg/day, i.p., for one month, beginning at the 16th week) diabetic rats (n=5). Malondialdehyde (MDA, nmol/mg protein) and glutathione (GSH, µmol/g tissue) levels were determined by spectrophotometry in aorta, pancreas, liver, brain, lung, kidney, heart and spleen. All values are expressed as mean±s.e. Statistically analysis of the data was performed using ANOVA followed by Tukey- Kramer multiple comparisons test. $P<0.05$ was considered as statistically significant.

Results : 1. MDA levels were significantly higher in aorta ($p<0.001$) and lower in kidney ($p<0.01$) and heart ($p<0.001$) and there were no significant differences in pancreas, liver, brain, lung and spleen of diabetic versus control rats. AICA-treatment normalized the decreased MDA levels in kidney and heart whereas not affected the increased MDA level in aorta of diabetic rats. 2. GSH levels were significantly lower in all tissues of diabetic used present study versus control rats ($p< 0.001$, 0.05, 0.001, 0.001, 0.001, 0.001, 0.001 and 0.05 respectively) and AICA treatment significantly increased all of the decreased GSH level of diabetic versus untreated diabetic control rats ($p< 0.001$, 0.05, 0.01, 0.01, 0.05, 0.001, 0.001 and 0.05 respectively).

Conclusion : Our results suggest that experimental diabetes can produce changes on the oxidant/antioxidant system not only in pancreas but also in aorta, liver, brain, lung, kidney, heart and spleen and AICA treatment, at least in part, could improve the changes on the oxidant/antioxidant system in diabetes.

PS 113
Homocysteine

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HOMOCYSTEINE RELATED GENE VARIANT IS NOT ASSOCIATED WITH ISCHAEMIC HEART DISEASE.

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(Aims)Ischaemic heart disease (IHD) is a common macrovascular complication of type 2 diabetes mellitus (DM), which may cause premature death along with diabetic nephropathy. A C-T mutation at nucleotide position 677 of methylenetetrahydrofolate reductase gene has been reported to be associated with IHD in Caucasians. However, conflicting results have also been reported. To know its role in the pathogenesis of IHD, we investigated whether this mutation is associated with IHD in Japanese patients with type 2 DM.

(Materials and Methods)We genotyped 68 diabetic patients with IHD and 140 diabetic patients without IHD. The patients with IHD were diagnosed as having IHD by either the presence of a past history of myocardial infarction or of an angiographically documented coronary artery stenosis. The risk factors such as age, duration of diabetes, onset age of type 2 DM, systolic and diastolic blood pressure were not different between the IHD+ and IHD- groups. The genotypes were determined by PCR-RFLP method. (Results)This variant was not associated with IHD in Japanese patients with type 2 DM (Chi-squared=0.93, p=.150). Allele frequencies were not different between the two groups (Chi-squared=0.008, p=0.93)

(Conclusions)Unlike some previous reports on Caucasians, this variant is unlikely to play a major role in the development of IHD in Japanese with type 2 DM.

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METHYLENETETRAHYDROFOLATE REDUCTASE GENE POLYMORPHISM IS NOT ASSOCIATED TO DIABETIC NEPHROPATHY IN TYPE 2 DIABETES

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Aims The increased susceptibility of type 2 diabetic patients with nephropathy to atherosclerosis can be explained only in part by the association with risk factors such as hypertension and hyperlipidemia. Mild hyperhomocysteinemia has been identified as an indipendent risk factor for atherosclerotic vascular disease both in diabetic and non-diabetic subjects. The individuals homozygous for the 677C→T (677TT) mutated allele of the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene have a 30-50% increase of plasma homocysteine, when compared to individuals homozygous for the 677C (677CC) allele. The aim of this study is to evaluate if the presence of diabetic nephropathy in type 2 diabetic patients is associated to the 677C→T mutation. **Materials and Methods** MTHFR genotyping was performed on all type 2 diabetic patients with diabetic nephropathy attending the outpatient diabetic clinic (No. 94), and on 93 normoalbuminuric type 2 diabetic patients matched with the cases for sex, and duration of diabetes. Genomic DNA was amplified by PCR, cleaved by enzimatic digestion with *Hinf* I, and electrophoresed through a 10% nondenaturing polyacrylamide gel.

Results

Variable	nephropathic	normoalbuminuric
MTHFR Genotypes	**n. (%)**	**n. (%)**
677CC	33 (35)	40 (43)
677CT	42 (45)	39 (42)
677TT	19 (20)	14 (15)
Allelic frequency		
677C allele	0.574	0.639
677T allele	0.425	0.360

The 677T allelic frequency is not statistically different between the nephropathic group and the normoalbuminuric group (0.425 vs 0.360, p=0.36)

Conclusions Our present observations suggest that the 677C→T mutation of the MTHFR gene, which is known to cause mild hyperhomocysteinemia, is not a genetic factor for explaining the increased incidence of cardiovascular disease in type 2 nephropathic diabetic patients.

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MTHFR MUTATION AND SUBCLINICAL CAROTID IMPAIRMENT IN AGING NON DIABETIC WOMEN

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Aims: hyperhomocysteinemia is an independent vascular risk factor. Little is known about the relationship between a point mutation (C677T) in the gene encoding MTHFR, a key enzyme in homocysteine metabolism, and early stages of atherosclerosis. **Materials and Methods**: we investigated the relationship between plasma homocysteine concentration (Hcy), measured by HPLC, MTHFR mutation, evaluated by polymerase chain reaction followed by HinfI digestion, and carotid geometry in 120 healthy women aged 52-80 years with normal glucose tolerance after OGTT and normal albumin excretion rate. In all women we measured intima-media thickness (IMT), speed of systolic peak (SPS), end-diastolic speed (EDS) and resistance index of intracranial circulation (IR) by ultrasound high-resolution B-mode imaging. All data are expressed as mean±SE. **Results**: 22 women were homozygous for the mutation (Group A), 67 were heterozygous (Group B) and 31 were homozygous for the wild-type allele (Group C). The three groups were comparable for age, BMI, lipid pattern, presence of hypertension. Plasma Hcy did not differ in the three groups (10.3±1.6 vs 12.7±2.3 vs 10.05±1.3 μmol/l, p=NS). Group A had significantly higher IMT respect to groups B and C (0.26±0.08 vs 0.14±0.01 and 0.16±0.02 mm, p=0.03), and higher EDS (27.7±1.8 vs 22.8±0.9 and 20.8±1.6 cm/sec, p=0.02 vs Group C). No differences were observed in both SPS (76.9±5.6 in A, 72.8±2.6 in B and 69.0±4.3 cm/sec in C) and IR (0.70±0.01 in A, 0.7±0.09 in B, 0.69±0.02 in C). In a multivariate regression analysis, age and IMT were independently associated with MTHFR mutation (p< 0.005). **Conclusions**: MTHFR homozygosis for C677T mutation is associated with some ultrasound parameters of asymptomatic carotid impairment. These observations suggest that MTHFR mutation could be a marker of early stages of atherosclerotic disease.

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SERUM HOMOCYSTEINE LEVELS ARE ASSOCIATED WITH THE DEVELOPMENT OF (MICRO)ALBUMINURIA: FOLLOW-UP OF THE HOORN STUDY

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Aims: Microalbuminuria is a strong indicator of risk of future cardiovascular disease and renal dysfunction. Slightly increased levels of homocysteine (Hcy), an independent risk factor for atherothrombotic disease, have recently been found to be associated with the presence of (micro)albuminuria. It is, however, unknown whether increased Hcy levels precede the occurrence of (micro)albuminuria.

Materials and methods: Normoalbuminuric subjects (n=316; 50 with non-insulin-dependent diabetes mellitus [NIDDM]) of an age-, sex-, and glucose-tolerance-stratified sample of a population-based cohort study were investigated after a mean follow-up duration of 6.1 years. Development of (micro)albuminuria was defined as a mean albumin-to-creatinine ratio >2.0 mg/mmol at follow-up examination.

Results: The cumulative incidence of (micro)albuminuria was 14.0% among non-diabetic subjects and 22.7% among NIDDM patients. Age-, sex- and glucose-tolerance-adjusted logistic regression analyses showed development of (micro)albuminuria to be significantly associated with Hcy levels >19.0μmol/l as compared to Hcy levels <9.1 μmol/l (OR 5.08[1.12-23.00]) For Hcy levels of 9.1-14.0 and 14.1-19.0 μmol/l, the ORs were 1.21[0.48-3.04] and 1.83[0.63-5.30], respectively. Substituting Hcy levels as a continuous variable for categories of Hcy levels showed that a 5 μmol/l increase of the Hcy level was associated with a 1.33(0.93-1.89) increased risk of developing (micro)albuminuria. Additional adjustment for insulin resistance, blood pressure, body mass index, presence of cardiovascular disease, retinopathy, current smoking or creatinine clearance did not materially affect the results. Analyses performed in non-diabetic and diabetic subjects separately gave similar results among non-diabetic subjects. Among diabetic subjects, the association between Hcy level and (micro)albuminuria could not be estimated, because there was an insufficient number of diabetic subjects with high Hcy levels.

Conclusion: Hyperhomocysteinemia level is an independent determinant of the development of (micro) albuminuria among non-diabetic subjects, even after adjustment for creatinine clearance. We could neither prove nor reject an association between Hcy levels and the development of (micro)albuminuria among NIDDM subjects. These data suggest that homocysteine may play a pathophysiological role in the development of (micro)albuminuria.

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IMPROVED METABOLIC CONTROL AND SERUM TOTAL HOMOCYSTEINE LEVELS IN TYPE 2 DIABETES PATIENTS

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Background. A high serum total homocysteine (tHcy) level has been shown to be an independent risk factor for atherosclerosis and cardiovascular disease, also in type 2 diabetes. The relation between the degree of metabolic control and tHcy level in this group of patients has not been established. **Aims.** To assess the influence of improved metabolic control of diabetes on tHcy levels in type 2 diabetes. **Materials and methods.** The study subjects were divided into two groups: I - 11 type 2 diabetic patients, in whom insulin monotherapy was going to be initiated due to insufficient control with oral agents (mean age 65.2±4.7 yrs, BMI 26.3±2.4 kg/m^2, duration of diabetes 10.5±3.9 yrs); II (controls) – 7 well-matched, well-controlled type 2 diabetics treated only with insulin (mean age 64.3±3.9 yrs, BMI 26.9±3.0 kg/m^2, duration of diabetes 11.3±4.2 yrs). Subjects with known cardiovascular disease were excluded from the study. Fasting serum tHcy concentration was assessed by high-performance liquid chromatography at baseline and after 3 months of insulin treatment, which aimed at achieving the optimal metabolic control. **Results** are shown in the table.

	HbA_{1c} (%)		tHcy (µmol/l)		daily insulin (IU)	
group	I**	II	I*	II	I	II
baseline	9.6±1.3$^{\#\#}$	6.8±1.0	19.0±5.2$^{\#\#}$	14.3±3.6	0	59±11
after 3 months	7.8±1.1$^{\#}$	7.0±1.0	17.4±4.9$^{\#\#}$	14.9±4.0	54±8	61±10

**p<0.05 baseline vs after 3 months values; **p<0.01 baseline vs after 3 months values; $^{\#}$p<0.05 I vs II; $^{\#\#}$p<0.01 I vs II*

Conclusions. Improvement in metabolic control achieved with insulin was associated with slight, but statistically significant decrease in fasting tHcy levels in type 2 diabetic patients. Our findings suggest that insulin and the level of metabolic control may play role in homocysteine metabolism in diabetic patients.

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PLASMA HOMOCYSTEINE AND DEGREE OF INSULIN RESISTANCE IN SUBJECTS WITH TYPE 2 DIABETES.

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Increased plasma homocysteinaemia (Hcy) is an independent risk factor for coronary and peripheral artery diseases (CAD and PAD), and is associated with micro- and/or macrovascular complications in subjects with type 1 diabetes. Raised Hcy is also associated with increased urinary albumin excretion in type 1 and type 2 diabetes. **Aims**: To assess whether raised fasting plasma Hcy is associated with insulin resistance. **Subjects and Methods**: We used the *Homeostasis Model Assessment* (HOMA), a structural computer model of the glucose/insulin feedback system (*Diabetes Research Labs, Oxford*) to simultaneously assess β-cell function (%β; *NI=100%*) and insulin sensitivity (%S; *NI=100%*) in 61 subjects with type 2 diabetes. **Results**: Sex ratio (M:F) was 72:28, mean age (±1SD) 63.1±10.4 yrs, known diabetes duration 12.9±9.3 yrs, BMI 30.0±6.1 Kg.m^{-2}, %β 64.5±46.3, %S 42.2±20.7, and HbA_{1c} 8.9±2.15%. Insulin therapy was present in 46%. Past or current smoking prevalence was 43 and 18% respectively. Raised Hcy levels (>15.0 mmol.L^{-1}; *FPIA-IMx analyser, Abbott*) were present in 25% of subjects (*n*=16; ↑Hcy group), while Hcy levels within the normal range (>5.0<15.0 mmol/L) were measured in the remaining 46 subjects (≡Hcy group). Hcy in CAD[+] and CAD[-] subjects was 14.5±7.6 and 12.0±5.1 mmol/L respectively (*NS*). A 20% prevalence for both CAD and PAD was found in ≡Hcy subjects, while this figure rose to 44% in ↑Hcy subjects. There were no significant differences in age, BMI, WHR, smoking exposure, diabetes duration, fasting lipids, %β, %S and HbA_{1c} between ↑Hcy and ≡Hcy subjects. **Conclusions**: Increased fasting plasma homocysteinaemia is not associated with the degree of insulin resistance as measured with HOMA in subjects with type 2 diabetes.

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HOMOCYSTE(E)INE AND ENDOTHELIAL DYSFUNCTION IN UNCOMPLICATED IDDM PATIENTS

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Aim: Elevated homocysteine (Hcy) levels represent a known risk factor for macrovascolar disease High Hcy levels were found in IDDM patients with diabetic complications. We assessed the link among Hcy with other parameters of coagulative and endothelial status in IDDM patients. Materials and Methods:We evaluated 57 well-controlled non-hypertensive IDDM patients (age 32±7 yrs, duration of disease 11.5±6.5 yrs, HbA1c 7.4±1.1%) without diabetic complications, compared with 44 controls matched for age, sex and life-style. Results:

	IDDM	Controls	P value
HCY mmol/l	15.3±11	9.98±5	<0.05
Endothelin 1 (ET-1) fmol/ml	2.8±1.6	0.5±0.2	<0.01
C reactive protein (CRP) mg/l	1.6±1.8	0.7±0.4	<0.01
NEFA meq/l	0.55±0.3	0.27±0.1	<0.01
LogLp(a) mg/dl	1.31±0.5	0.53±0.3	<0.01
Phospholipids (PHO) mg/dl	233±32	206±28	<0.01
Fibrin monomers (FM) mg/l	19.6±11	8.6±5.5	<0.01
D-Dimers (D-Dim) ng/l	305±187	226±52	<0.05
Plasminogen (PLG) %	92±17	101±12	<0.01

IDDM patients,were characterized by: 1) higher Hcy plasma levels associated to an endothelial dysfunction (higher levels of ET-1 and CRP), 2) an alteration of lipid metabolism, (higher NEFA, Lp(a) and PHO levels); 3) a procoagulant state (higher levels of FM and D-Dim, reduction of PLG). Significative correlations were found in IDDM among Hcy with HbA1c (R=0.330, p<0.05), CRP (R=0.450,P<0.01), NEFA (R=0.450,P<0.01) and D-Dim (R=0.280, p<0.05). Conclusions: these data suggest that in uncomplicated IDDM patients hyperhomocysteine can be related to

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HOMOCYST(E)INE, SIALIC ACID AND THE VASCULAR ENDOTHELIUM IN LONGSTANDING TYPE 1 DIAETIC SUBJECTS. J.Gibney, U.Weiss, B.Turner, P.O'Gorman, G.Watts, J.Powrie, M.Crook, K.Shaw and M.Cummings. Queen Alexandra hospital, Portsmouth; St.James'hospital, Dublin; St.Thomas'hospital, London. **Aims**: Urinary albumin excretion rate (AER) represents an early stage in development of diabetic nephropathy and probably reflects widespread endothelial damage. Plasma homocyst(e)ine (HCY) is associated with endothelial dysfunction and is an independent risk factor for atherosclerosis. Serum sialic acid (SSA) AND C-reactive protein (CRP) are markers of vascular inflammation and are both predictive of atherosclerosis. We wished to assess whether these variables reflect the same pathological process. **Materials and methods**: 88 longstanding (mean duration – 25 years) type 1 diabetic subjects were studied on 2 occasions 5 years apart. Retinopathy, SSA, AER and lipid profile were assessed at both timepoints. Evidence of coronary artery disease (ECG and Rose questionnaire), HCY and CRP were assessed at the later timepoint only. Statistical analyses were performed using backward stepwise logistic regression models. **Results**: Retinopathy, AER and SSA all progressed independently of each other. Progression of both retinopathy and AER were independently predicted by baseline AER only (p=0.02 and 0.0001 respectively). Progression of SSA was independently predicted by apoB only (p<0.005). Both retinopathy and coronary artery disease at the later timepoint correlated with plasma HCY only. This correlation was independent of serum creatinine and AER. The level of HCY was determined by gender (increased in males, p=0.005) and serum creatinine (p<0.0001). CRP correlated independently with age, gender (increased in males, p<0.05 for both) and SSA (p<0.02). **Conclusions:** Vascular inflammation assessed by SSA and CRP is influenced by dyslipidemia only and progresses independently of microvascular disease. Plasma HCY correlates with both microvascular and macrovascular complications in type 1 diabetic subjects and may have a causative role in their development. This is a potential explanation for the increased progression of retinopathy and atherosclerosis which are associated with early renal dysfunction in diabetic subjects.