OP 1

Glycaemic control of type 2 diabetes

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Exenatide achieved equivalent glycaemic control to insulin glargine, with weight reduction and less nocturnal hypoglycaemia, in metformin and sulfonylurea-treated type 2 diabetes

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Background and Aims: Clinical studies have shown that exenatide improves glycaemic control, and is associated with weight reduction, reduced postprandial glucose excursions, and a low incidence of hypoglycaemia in type 2 diabetes patients inadequately controlled by MET and/or SFU. The addition of basal insulin is currently common practice when these orals fail, but can be associated with increased hypoglycaemia, inadequate postprandial glucose control, and weight gain. The primary aim of this trial was to determine whether exenatide can be used as an alternative to basal insulin (insulin glargine) for type 2 diabetes patients sub-optimally controlled with MET + SFU.
Materials and Methods: 82 outpatient study centers in 13 countries participated in this 26-week trial. Type 2 diabetes patients (HbA_{1c} 7.0–10.0%) were randomized to exenatide (5 µg BID for first 4 wks, 10 µg BID remainder of study, n=283) or insulin glargine QD (titrated to FBG<5.6 mmol/L, n=268), adjunctive to pre-existing MET + SFU. The primary endpoint was the change in HbA_{1c} from baseline to Week 26.
Results: Baseline HbA_{1c} (mean±SE) was 8.2 ± 1.0% for exenatide and 8.3 ± 1.0% for insulin glargine. At endpoint, exenatide and glargine resulted in similar reductions in HbA_{1c} (exenatide: –1.0 ± 0.1%, glargine: –1.1 ± 0.1%; 95% CI for exenatide-glargine difference = –0.1 to 0.2%). A similar proportion of patients in both groups achieved HbA1c ≤7% at endpoint (exenatide: 46%, glargine: 48%). Both treatments caused reductions in fasting glucose; however, glargine did so to a significantly greater extent (exenatide: –1.2 ± 0.2 mmol/L, glargine: –2.9 ± 0.2 mmol/L; p<0.0001). Following a test meal, postprandial glucose excursions were diminished in exenatide patients (incremental $AUC_{0–4\ hrs}$: –0.3 ± 1.0 mmol-hr/L), while glargine did not reduce postprandial glucose excursions (incremental AUC: +7.0 ± 1.2 mmol-hr/L). Body weight changes were –2.3 ± 0.2 kg for exenatide vs +1.8 ± 0.2 kg for glargine (p<0.0001). Rates of symptomatic hypoglycaemia were similar between treatments, but nocturnal hypoglycaemia was lower for exenatide (0.9 ± 0.4 vs. 2.4 ± 0.4 events/patient year, p<0.0001). The most common adverse event among exenatide patients were nausea, generally reported as mild to moderate episodes with decreasing incidence during the study. 81% of exenatide vs 90% of glargine patients completed the study; 9.5% of exenatide vs 0.7% of glargine patients withdrew due to adverse events.
Conclusion: Fixed dose exenatide achieved similar improvements in overall glycaemic control to glargine titration in patients with long-standing type 2 diabetes inadequately controlled by MET + SFU. Glargine predominantly reduced fasting plasma glucose, while exenatide was associated with a lower incidence of nocturnal hypoglycaemia, better postprandial glucose control, and progressive weight reduction. These findings support the potential use of exenatide prior to the addition of starter basal insulin for type 2 diabetes patients sub-optimally controlled with oral combination therapy.
Sponsored by Eli Lilly and Company and Amylin Pharmaceuticals, Inc.

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Clinical site experience comparing insulin glargine with exenatide treatment in type 2 diabetic patients

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Background and Aims: Exenatide is a new anti-diabetic agent under clinical investigation that may improve diabetes control by enhancing glucose-dependent insulin secretion and glucagon suppression without weight gain. In a *post hoc* analysis, we report the individual responses during a 30 week open-label phase III clinical trial comparing the effects of exenatide and insulin glargine in type 2 diabetic patients inadequately controlled (HbA1c >7,5) on combination therapy with sulphonylurea and biguanide.
Materials and Methods: Fifty-five patients were randomized to exenatide (n=29, 10 mcg twice daily, baseline data: 59% female, 61,3 ± 9,5 years and 74,5 ± 10 kg) or insulin glargine (n=26, baseline data: 31% female, 61,4 ± 8,4 years and 81,3 ± 15,2 kg) at two Spanish clinical sites. Insulin glargine was initiated with 10 IU/day using a forced titration to achieve a target goal of fasting blood glucose <5.6 mmol/L (mean dose at endpoint = 13 ± 4 IU). Measures included change in HbA1c, body weight, seven-point blood glucose (BG) measurements, and safety and tolerability. 65% of exenatide and 88% of insulin glargine patients completed the study. Withdrawal reasons: adverse events: 4 exenatide, 1 glargine; protocol violation: 5 exenatide, 2 glargine; patient's decision: 1 exenatide
Results: From baseline to endpoint (see enclosed table), there was a change in Hb1Ac and body weight of –0,76% and –1,1 kg in the exenatide group and –0,96% and +1,0 kg in the glargine group, respectively. 41% of exenatide patients and 34% of glargine patients achieved HbA1c ≤ 7% at endpoint. Sixty-two adverse events (n=22) were recorded in the exenatide group (39 events of nausea, 22 of hypoglycemia and 5 of vomiting) versus 42 events (n=12) in the glargine group (40 events of hypoglycemia and 2 of vomiting). The clinician and the patient scored only 3 exenatide emergent adverse events (nausea) and 0 glargine events as severe.
Conclusion: Both treatments reduced HbA1c after 26-weeks of treatment. A flatter 7-point blood glucose curve and greater weight reduction was observed in the exenatide group at the end of the study. The most common side effects were mild nausea in the exenatide group and mild-to-moderate hypoglycemia in the insulin glargine group.

	Exenatide Group: Baseline	Exenatide Group: Endpoint	Insuline Glargine Group: Baseline	Insuline Glargine Group: Endpoint
Hb A1c (%)	8,08	7,32	8,45	7,49
Body weight (kg)	74,5	73,4	81,3	82,3
Pre-breakfast BG (mmol/L)	7,80	7,57	8,11	8,01
2 h post-breakfast BG (mmol/L)	8,04	8,05	10,03	9,94
Pre-lunch BG (mmol/L)	7,34	7,16	8,08	8,06
2 h post-lunch BS (mmol/L)	8,78	8,65	9,21	9,14
Pre-dinner BS (mmol/L)	7,95	7,77	8,29	8,16
2 h post-dinner BS (mmol/L)	8,18	7,98	10,09	9,92
03:00 AM BS (mmol/L)	7,44	7,44	8,44	8,42

Support: Eli Lilly and Company and Amylin Pharmaceuticals, Inc.

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Basal-only or premixed analogue insulin therapy: which has better HbA_{1c} control among patients with type 2 diabetes? A large naturalistic longitudinal cohort study

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Background and Aims: Over last several years, many patients with type 2 diabetes received basal-only analogue insulin therapy. However, there is no large-scale empirical study that examined whether basal-only analogue insulin therapy has equivalent or better therapeutic effects in reducing Hemoglobin A1c (HbA1c), compared to premixed analogue insulin therapy. Our study aims to fill this information gap through comparing the therapeutic effects of Lispro Mix 75/25 (LM), a premixed analogue insulin therapy, and Glargine (GL), a basal-only analogue insulin therapy, in reducing HbA1c among patients with type 2 diabetes in a large and naturalistic setting.
Materials and Methods: A naturalistic longitudinal cohort study design was used with a large national electronic medical records database (GE medical record database) that had 112,862 type 2 diabetics for a period between 1998 and 2004. From the database, we selected and analyzed medical records for all patients who had type 2 diabetes, multiple HbA1c records and used only LM (n=1,569) or only GL (n= 7,036) between 1998

and 2004. Subjects' HbA1c results were categorized into baseline (at 1st prescription for LM or GL), and 3, 6, and 9 months after the baseline (Q1, Q2, Q3). A propensity-score group matching method was used to reduce patients' demographic and clinical heterogeneities in age, gender, baseline HbA1c, percent of patients with diabetic complications across LM and GL cohorts. The mean reductions of HbA1c results from baseline to Q1, Q2 and Q3 were then calculated for and compared between LM cohort and GL cohort.
Results: LM and GL cohorts had similar gender distribution and baseline HbA1c levels. But the mean ages of the two cohorts were different (LM: 62, GL: 58). The mean reductions of HbA1c from baseline to Q1, Q2, and Q3 were presented for and compared across LM and GL cohorts in Table 1. In general, the mean reductions of HbA1c in LM cohort were larger than that in GL cohort with absolute differences ranging from 0.1 to 0.6 in the unmatched analyses and from 0.6 to 0.7 in the group-matched analyses.
Conclusion: In this large naturalistic cohort study, premixed analog insulin (Lispro Mix 75/25) is associated with a greater reduction of HbA1c from baseline to Q1, Q2 and Q3, compared to basal-only analogue insulin therapy (Glargine).

Table 1. Comparison of the mean reductions of HbA1c results across cohorts

Duration	Mean HbA1c Reductions in Lispro Mix Cohort	Mean HbA1c Reductions in Glargine Cohort	Differences of Mean HbA1c Reductions (LM-GL)
Without Group Matching			
Baseline to Q1	−0.9	−0.7	−0.1
Baseline to Q2	−0.9	−0.5	−0.4
Baseline to Q3	−1.0	−0.4	−0.6
With Group Matching			
Baseline to Q1	−2.0	−1.4	−0.6
Baseline to Q2	−2.4	−1.7	−0.7
Baseline to Q3	−2.4	−1.7	−0.6

Note: All mean changes of HbA1c are statistically significant with p-value <0.001

Support: Eli Lilly and Company

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Insulin glulisine provides superior postprandial glucose control with less nocturnal hypoglycaemia compared with regular human insulin in subjects with type 2 diabetes
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Background and aims: This 26-week, multinational, multicentre, controlled, open, randomized, parallel trial compared the efficacy and safety of insulin glulisine (GLU) and regular human insulin (RHI), both in combination with NPH insulin.
Materials and methods: Subjects with Type 2 diabetes (T2DM; n=892) received sc injections of GLU (n=448) 0–15 min before meals or RHI (n=444) 30–45 min before meals, plus twice-daily NPH insulin. NPH insulin doses were titrated to blood glucose (BG) targets 5.0–6.7 mmol/L (90–121 mg/dL) while avoiding hypoglycaemia; bolus insulin doses were titrated to 2-h postprandial (PP) BG targets 6.7–8.9 mmol/L (121–160 mg/dL). A standardized test meal was performed at baseline and endpoint, and fasting plasma glucose (FPG) measured; standard bolus insulin (0.15 U/kg) was given with the usual NPH insulin dose, then a 600 calorie meal; plasma glucose (PG) was measured 1 h and 2 h after the start of the meal.
Results: Mean baseline characteristics were similar in both groups (age 59.9 ± 9.3 yrs; BMI 31.2 ± 5.0 kg/m^2; HbA_{1c} 7.5 ± 0.9%; diabetes duration 13.5 ± 7.4 yrs; OAD use: 33.7% GLU vs 33.5% RHI). At baseline, most subjects injected short-acting insulin 3 times daily, and basal and mixed insulins 1–2 times daily. Baseline to endpoint change in HbA_{1c} was similar for GLU and RHI (adjusted mean change −0.31 vs −0.35%). At endpoint, BG excursions were significantly lower with GLU than RHI at breakfast, dinner and daily, but similar at lunch (Table). During the test meal, if FPG was similar in both groups, PP PG values and PG excursions were significantly lower with GLU vs RHI (2-h PP PG values: 14.14 vs 15.28 mmol/L [254.5 vs 275.0 mg/dL]; p=0.0025). Prandial glucose excursions at 1 h and 2 h post-test meal were significantly lower with GLU vs RHI at endpoint (3.99 vs 4.59 mmol/L [71.8 vs 82.6 mg/dL]; p=0.0151 and 4.87 vs 6.03 mmol/L [87.7 vs 108.5 mg/dL]; p=0.0002, respectively). The superior PP glucose control with GLU was not associated with increased hypoglycaemia; no noteworthy differences occurred between groups in the frequencies and monthly rates of all symptomatic hypoglycaemia. While not significant, severe hypoglycaemia rates were lower with GLU vs RHI (1.3 vs 3.2%; 0.004 vs 0.017 episodes/subject-month). Month 4 to study end covers a period when subjects were already acclimatized to treatment. Therefore, to eliminate potential bias due to learning effect, this was considered the main interest period. During this time, subjects with ≥1 episode of nocturnal hypoglycaemia and the monthly nocturnal hypoglycaemia rate were lower with GLU vs RHI (9.1 vs 14.5%; p=0.0290; 0.06 vs 0.11 episodes/subject-month; p=0.0309). Analyses in 6-h intervals over 24 h corroborated these results: the percentage of subjects with ≥1 symptomatic hypoglycemic episode was significantly lower from 00:00–06:00 (8.9 vs 14.1%; p=0.0316).
Conclusions: GLU provides superior PP glucose control vs RHI in subjects with T2DM, and is associated with a lower rate of nocturnal hypoglycaemia.

Mean BG excursions at endpoint from self-monitored daily profiles	GLU n	GLU Adjusted mean, mmol/L (mg/dL)*	RHI n	RHI Adjusted mean, mmol/L (mg/dL)*	p value for treatment effect
Breakfast	426	1.00 (18.0)	431	1.57 (28.3)	0.0001
Lunch	425	1.69 (30.4)	428	1.63 (29.3)	0.7340
Dinner	425	0.92 (16.6)	425	1.30 (23.4)	0.0103
Average daily	428	1.21 (21.8)	431	1.51 (27.2)	0.0010

*Adjusted means from ANCOVA model; BG=blood glucose; GLU=insulin glulisine; RHI=regular human insulin; intent-to-treat population

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Insulin detemir results in less weight gain than NPH insulin when added to oral agents in type 2 diabetes, with this advantage increasing with baseline obesity
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Background and Aims: The initiation or intensification of insulin therapy often results in weight gain, which may be problematic in already overweight patients with type 2 diabetes. However, in patients with type 2 diabetes there has been significantly less weight gain in association with the basal insulin analogue insulin detemir when compared with NPH insulin. The present analysis sought to determine whether the extent of this apparent advantage is dependent on patients' body mass index (BMI) at the initiation of treatment, comparing insulin detemir with NPH insulin as add-on therapy to OADs in a treat-to-target protocol.
Materials and Methods: In a 26-week, multicentre, randomised, parallel-group study, 476 insulin-naïve patients with type 2 diabetes inadequately controlled by OADs received addition of insulin detemir or NPH insulin twice daily (morning and evening). Insulin dose was aggressively titrated using a predefined algorithm over a 24-week active treatment period, designed to regain excellent glycaemic control. Weight change from baseline was analysed as a function of baseline BMI, and was also stratified by categories of BMI.
Results: Mean HbA1c decreased by 1.84% and 1.90% points with insulin detemir and NPH insulin, respectively, to endpoint values of 6.58% and 6.46% (ns). Regardless of baseline BMI, patients gained less weight with insulin detemir than with NPH insulin. With increasing baseline BMI, patients gained less weight with insulin detemir (p=0.01 for the linear regression of change in body weight vs. baseline BMI); this relationship was not found for NPH insulin (ns). These patterns were also apparent with stratification of mean weight gain by category of entry BMI as demonstrated in the table below.
Conclusion: The discrepancy in weight gain favouring insulin detemir over NPH insulin increases with baseline BMI when these insulins are added to OADs in a treat-to-target protocol. Insulin detemir may therefore offer a weight advantage over NPH insulin, especially in overweight or obese people with type 2 diabetes initiating insulin therapy.

Baseline BMI (kg/m²)	Insulin detemirN	Insulin detemir Weight gain (kg)	NPH insulin N	NPH insulin Weight gain (kg)
≤25	35	2.25	36	3.15
>25–27	34	1.81	39	2.73
>27–29	55	1.10	37	3.08
>29–31	42	0.54	50	2.79
>31	69	0.63	76	2.60

Support: Novo Nordisk

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Insulin glargine improves metabolic parameters in well-controlled (HbA_{1c}<7%) patients with type 2 diabetes previously on an intensive conventional insulin therapy (ICT) with NPH insulin

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Background and Aims: To date, continuous blood glucose monitoring system (CGMS; Medtronic Minimed) has been used predominantly to determine blood glucose (BG) profiles and the incidence of hypoglycaemia in patients with type 1 diabetes. In this multicentre, single-arm, 10-week study, patients with type 2 diabetes with a mean HbA_{1c} < 7% were switched from an intensive conventional insulin therapy (ICT) with NPH insulin to insulin glargine. The aim of the study was to determine whether these patients could further improve their glycaemic control with insulin glargine.
Materials and Methods: In addition to HbA_{1c} and fasting blood glucose (FBG), continuous BG profiles were analysed with CGMS. At the start of the study, patients (mean age 59.2 years; mean duration of diabetes 12.7 years; mean body mass index [BMI] 31.7 kg/m²) continued treatment with NPH insulin for 2 weeks, after which BG profiles were determined for 72 hours using CGMS (Baseline Visit, BV). Patients were then switched to insulin glargine and, following 8 weeks of therapy, CGMS measurements were repeated (Last Visit, LV). Data were analysed according to the previous number of NPH insulin injections: once daily, twice daily or more than twice daily. CGMS data were used to compare number of patients documenting hypos by patient diaries or BG values <60 mg/dL in 8-point profiles.
Results: In the 247 patients who had previously received NPH insulin once daily, mean HbA_{1c} levels significantly decreased from 6.87% at baseline to 6.66% at endpoint (p <0.001). For the 112 patients previously receiving twice daily NPH insulin, the mean HbA_{1c} levels decreased from 6.95% at baseline to 6.69% at endpoint (p <0.001). Only eight patients received >2 NPH injections/day; for these patients, HbA_{1c} decreased from 7.25% to 6.89% over the study period (p <0.177). FBG levels also decreased significantly (p <0.001) in two previous NPH insulin injection groups from baseline to endpoint (once daily: 138 to 128 mg/dL; twice daily: 143 to 131 mg/dL) and for > twice daily: 154 to 140 mg/dL (p=0.341). The percentage of BG values lower than 60 mg/dL, as determined by CGMS, remained almost unchanged from baseline to endpoint, regardless of the previous number of NPH insulin injections (once daily: 1.8 ± 3.1% to 2.2 ± 4.1%, p=0.101; twice daily: 2.2 ± 4.3% to 2.3 ± 4.2%, p=0.759; more than 2 injections daily: 1.8 ± 3.3% to 1.6 ± 2.6%, p=0.399). The numbers of patients documenting hypoglycaemic episodes by patient diaries or BG values <60 mg/dL in 8-point profiles changed from baseline to endpoint as follows: once-daily NPH: 62 to 65 patients; twice-daily NPH: 37 to 30 patients and for more than twice-daily NPH: 3 to 2 patients.
Conclusion: Patients with type 2 diabetes who were well controlled on ICT with NPH insulin further improved their glycaemic control when switched to insulin glargine therapy, regardless of the number of NPH insulin injections in their previous treatment regimen. There were no increases in the number of patients experiencing BG values < 60 mg/dL, as measured by CGMS, when switched to insulin glargine therapy.
This study was supported by sanofi-aventis.

OP 2
Inhibiting angiotensin, aldosterone and nephropathy

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Remission of nephrotic range albuminuria reduces risk of end-stage renal disease and improves survival in type 2 diabetic patients

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Background and Aims: Among patients with diabetic nephropathy those with nephrotic range albuminuria (NRA) are known to have the poorest renal and cardiovascular prognosis. We evaluated the impact of remission of NRA, defined as persistent albuminuria >2.5g/24-hrs, on end-stage renal disease (ESRD) and mortality in type 2 diabetic patients with nephropathy, followed in a prospective observational study.
Materials and methods: From a cohort of all type 2 diabetic patients with nephropathy followed for at least 3 at Steno Diabetes Center (n=227) we included in the present observational follow-up study all patients with NRA (n=79 (62 males, 17 females)). Patients were followed from onset of NRA until death or January 2005; Age (mean(SD)) 60(8) years, diabetes duration 14(7) years.Remission of NRA was defined as albuminuria <0.6g/24 h sustained for at least one year. Principal endpoints: ESRD or death. Secondary endpoint: Rate of decline in GFR (^{51}Cr-EDTA technique).
Results: NRA occurred in 79 of the 227 patients. Duration of follow-up after onset of NRA was 6.5 (2–20) years, and remission was induced in 20 patients (25%). All patients who obtained remission were treated with either an ACE-inhibitor or an angiotensin II receptor blocker at the end of follow-up as compared with only 46 (78%) of the patients who did not obtain remission (p<0.05). Remission lasted 4.1(1–10) years and only 3 patients relapsed from remission. At end of follow-up, 30% of 20 patients with remission had reached the composite endpoint of ESRD or death (2 ESRD and 4 deaths) in contrast to 66% of 59 patients without remission (16 ESRD and 23 deaths) (p<0.01). Cox-regression analysis revealed that remission was associated with a risk reduction of 66% (95%CI: 13–90) of reaching the composite end-point of ESRD or death and of 69% (95%CI: 21–88) for death alone. In these models, male gender, higher age, and systolic blood pressure at onset of NRA were also independently associated with an increased risk of ESRD and death. The rate of decline in GFR tended to be lower in the patients who obtained remission 5.4 (1.0) ml/min/year as compared with 8.4 (1.1) ml/min/year in patients without remission, p=0.08.
Conclusions: Nephrotic range albuminuria occurs frequently in type 2 diabetic patients with nephropathy, however long-term remission can be obtained in a sizable fraction by aggressive antihypertensive treatment, in particular by blocking the renin-angiotensin system. Remission of nephrotic range albuminuria is associated with a slower progression in nephropathy and substantially improved survival.
Support: Danish Diabetes Association

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Enhanced renoprotective effects of ultra high doses of irbesartan in patients with type 2 diabetes and microalbuminuria

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Background and Aims: Despite the proven benefit of treatment with irbesartan in preventing diabetic nephropathy the optimal renoprotective dose remains unknown. We evaluated if irbesartan have additional antiproteinuric effects when given in ultra high doses exceeding the currently recommended maximum dose of 300 mg in type 2 diabetic patients with microalbuminuria.
Materials and Methods: This double-masked randomized cross-over trial included 52 (41 males) hypertensive type 2 diabetic patients with microalbuminuria on ongoing antihypertensive medication. At inclusion, previous antihypertensive treatment was discontinued and replaced with bendroflumethiazide 5 mg o.d. for the entire study. Following two months wash-out(baseline), patients were treated randomly with irbesartan 300, 600 and 900 mg o.d., each dose for two months. End-points evaluated at the end of each study period included urinary albumin excretion rate(UAE, mean of three 24-hrs collections), 24-hrs blood pressure(ABP); and GFR(51Cr-EDTA).

Results: Baseline values were: 24-hrs UAE [geometric mean(95%CI)] 134 (103 to 170) mg/24-hrs, ABP[mean(SD)] 140 (10)/77 (7) mm Hg and GFR 103 (19) ml/min/1.73 m^2.
All doses of irbesartan significantly reduced UAE, ABP, and GFR from baseline. Reductions in UAE from baseline were 52 (46 to 57), 49 (43 to 54) and 59 (54 to 63)% with increasing doses of irbesartan ($p<0.01$).
UAE was reduced significantly more by irbesartan 900 mg compared with lower doses with an additional reduction in UAE of 15 (2 to 26)% by irbesartan 900 vs. 300 mg ($p=0.02$). The additional reduction in albuminuria by irbesartan 900 vs. 300 mg was more pronounced in patients with levels of UAE during irbesartan 300 mg above vs. below the median (31 (18 to 42) vs. –9 (–25 to 6)%, respectively ($p<0.05$)).
With increasing doses of irbesartan systolic-ABP was reduced from baseline by 8 (4 to12), 9 (5 to13) and 9 (5 to13) mm Hg (NS), and diastolic-ABP by 6 (4 to7), 7 (6 to 9), and 7 (6 to 9) mm Hg (NS).
Conclusion: Ultra-high doses of irbesartan (900 mg o.d.) is generally safe and offer additional renoprotection independent of changes in systemic blood pressure and GFR in comparison to the currently recommended dose of 300 mg.
The study was supported by an unrestricted study grant from Sanofi-Aventis, who also provided the study medication.

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Possible involvement of local aldosterone production by renal mesangial cells in diabetic nephropathy
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Background and Aims: Aldosterone is classically produced by the zona glomerulosa cells of the adrenal cortices. Systemic aldosterone plays an important role in the development of the microvascular disease and glomerular damage of the kidney in patients with diabetes mellitus and hyperlipidemia. Here, we investigated the possibility of local production of aldosterone in the kidney, using human primary glomerular mesangial cells.
Materials and Methods: Primary human mesangial cells (product code: cc-2559) were obtained from Bio Whittaker, Inc. The cells were cultured in MCDB131 medium containing 5.5 mM glucose, 5% fetal calf serum, 50 μg/ml gentamycin sulfate and 50 ng/ml amphotericin-B in a humidified 5% CO_2 incubator at 37 °C. Studies were performed between passages 4 and 7.
Results: These cells produced both pregnenolone and aldosterone measured by specific radioimmunoassay and/or GC/MS methods. The production of both steroids was significantly stimulated by treatment with LDL, while angiotensin II had a synergistic effect. ACTH and (Bu)2 cAMP, on the other hand, failed to stimulate aldosterone production by these cells, suggesting that the local production of this steroid by mesangial cells is regulated differently from that of adrenal zona glomerulosa cells. Mesangial cells expressed the mRNA of the LDL receptor and steroidogenic enzymes such as P450scc, 3β-HSD, 21-hydroxylase and CYP11B2. Mesangial cells also expressed the mRNA of mineralocorticoid receptor.
Conclusion: These data demonstrated that the human mesangial cells are an aldosterone-producing tissue in which LDL plays a major regulatory role. Mesangial cells also possess mineralocorticoid receptor. Thus, it is suggested that there may be existed some interaction between locally produced aldosterone and mineralocorticoid receptor located inside the mesangial cells. The human renal mesangial endocrine system may contribute to local aldosterone concentration in the renal glomerulus independently of the systemic renin-angiotensin-aldosterone system and may participate in the development and progression of glomerular damage in several pathologic conditions such as diabetic nephropathy.

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Beneficial impact of spironolactone in diabetic nephropathy
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Background and Aims: Aldosterone has been suggested to play a role in the initiation and progression of diabetic nephropathy. Currently recommended treatment with ACE inhibitors or angiotensin II receptor blockers (RAS-blockade) does not suppress circulating aldosterone sufficiently. We therefore aimed to evaluate the short-term effect of aldosterone antagonism with spironolactone on albuminuria and blood pressure in diabetic nephropathy.
Materials and Methods: We performed a double-masked, randomized, placebo-controlled, cross-over trial. Twenty type 1 diabetic patients with diabetic nephropathy were treated in random order with spironolactone 25 mg once daily and matched placebo for two months respectively. Study medication was added to ongoing antihypertensive treatment, including RAS-blockade. After each treatment period, albuminuria (based on three 24-hour urine collections), 24-hour blood pressure and GFR were determined.
Results: Spironolactone on top of ongoing antihypertensive treatment, induced a 30% (95% CI: 17–41) reduction in albuminuria from (geometric mean (95% CI)) 831 (624–1106) mg/24-hour on placebo treatment ($p<0.001$), and a reduction in fractional albumin clearance of 35% (20–46, $p<0.001$). Daytime blood pressure was reduced by (mean reduction (95% CI)) 10 (2–18)/5 (1–8) mm Hg ($p<0.02$), but night blood pressure remained unchanged. There was an insignificant reversible reduction in GFR during treatment with spironolactone.
Conclusion: Our results suggest that spironolactone treatment on top of recommended antihypertensive treatment may offer additional renoprotection in type 1 diabetic patients with diabetic nephropathy.
Support: The Danish Diabetes Association

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Beneficial effects of adding spironolactone to recommended antihypertensive treatment in diabetic nephropathy
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Aims: To evaluate the safety and short-term effect of adding spironolactone to conventional antihypertensive treatment including diuretics and maximally recommended doses of an ACE-I or an angiotensin II receptor blocker(ARB) on albuminuria and blood pressure in type 2 diabetic patients with nephropathy.
Materials and Methods: Twenty-one type 2 diabetic patients with nephropathy were enrolled in a double-masked, randomized cross-over study. Patients were treated in random order with spironolactone 25 mg o.d. and matched placebo for eight weeks respectively, on top of ongoing antihypertensive treatment including diuretics and maximally recommended doses of an ACE-I and/or an ARB. At the end of each treatment period albuminuria, 24-hour ambulatory blood pressure(ABP) and glomerular filtration rate(GFR) were determined.
Results: During addition of placebo values were: Albuminuria [geometric mean(range)]: 1566 (655 to 7762) mg/24-hrs, ABP [mean(SE)]: 138(3)/71(1) mmHg, GFR 74(6)ml/min/1.73 m^2. During addition of spironolactone albuminuria was reduced by 33(95%CI: 25–41)% ($p<0.001$), fractional clearance of albumin by 40(24 to 53)% ($p<0.001$), and ABP was reduced by 6(2 to 10) mmHg systolic and 4(2 to 6) mmHg diastolic($p<0.001$ for both). Change in albuminuria did not correlate to change in systolic-ABP ($r=0.19$, $p=0.42$) or diastolic-ABP ($r=0.01$, $p=0.96$). Spironolactone treatment induced an insignificant reversible reduction in GFR of 3 (–0.3 to 6) ml/min/1.73 m^2($p=0.08$). One patient was excluded due to hyperkalemia. Otherwise treatment was well tolerated.
Conclusion: Our study suggests that spironolactone safely adds to the reno- and cardiovascular protective benefits of treatment with maximally recommended doses of ACE-I and ARB by reducing albuminuria and blood pressure in type 2 diabetic patients with nephropathy.
Support: The Danish Diabetes Association

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Anti-proteinuric effect of spironolactone in type 2 diabetic nephropathy: a randomized placebo-controlled study
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Background and Aims: Aldosterone has been implicated in the progression of proteinuria and renal dysfunction. Moreover, aldosterone escape during ACE-inhibition has been reported. We therefore evaluated effects of spironolactone (Spir) on renal functon, albuminuria and blood pressure (BP) in type 2 diabetic patients with diabetic nephropathy (DN) already on ACE inhibition or angiotensin II receptor blockade (ARB).
Materials and Methods: In a masked, parallel-group trial, type 2 diabetic patients with DN were randomized for Spir or matched placebo's (Plac).

Follow-up lasted 1 year. BP, urinary albumen-creatinine ratio (UACR), and biochemical parameters were measured at 3 monthly intervals.The starting dose of Spir was 50 mg daily. This dose was reduced to 25 mg if hyperkalemia (> 5.5 mmol/) developed.
Results: 29 patients were allocated to Spir and 30 to Plac. Five patients in Spir and 1 in Plac group had to be withdrawn shortly after randomisation because of development of hyperkalemia. Compared to normokalemic patients they had higher ($p < 0.001$) serum concentrations (mean ± SD) of creatinine (171 ± 63 vs 94 ± 37 micromol/l) and potassium (4.7 ± 0.3 vs 4.2 ± 0.2 mmol/l). Remaining subjects of Spiro and Plac group had similar age (57 ± 10 and 56 ± 11 yrs), body mass index (32 ± 6 and 30 ± 5 kg/m^2), and glycoHb (8.2 ± 1.5 and 8.2 ± 1.4%). Baseline values of BP, UACR (median and IQ range), creatinine, and potassium in Spir and Plac group and their percentage changes (1 year average values) are given below.

	Spir	Plac	Spir % change	Plac % change	p-value
SBP, mmHg	141 ± 16	147 ± 14	–4.5 ± 8.7	–0.1 ± 6.8	0.04
DBP, mmHg	79 ± 7	81 ± 6	–3.5 ± 7.2	1.2 ± 6.6	0.09
UACR, mg/mol	79 (39–161)	61 (28–105)	–44 ± 47	14 ± 72	0.002
Creatinine, micromol/l	80 ± 23	108 ± 42	20 ± 13	9.2 ± 12.1	0.002
Potassium, mmol/l	4.1 ± 0.3	4.2 ± 0.4	9.6 ± 8.3	3.2 ± 10.1	0.02

In the Spir group changes in UACR were correlated with changes in serum creatinine ($r = -0.44$, $p = 0.03$) and systolic BP ($r = 0.46$, $p = 0.02$).
Conclusions: Addition of Spir on top of ACE inhibition or ARB produces a marked reduction of UACR in DN. However, this effect is at cost of faster deterioration of renal function within the one year time frame of our study. Close monitoring of serum potassium during initiation of spironolactone therapy is required in patients with impaired renal function.

OP 3
Mechanisms in cardiac complications

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Metformin activates AMP-activated protein kinase (AMPK) in the rat heart and reduces myocardial infarct size 24 hours later
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Background and Aims: Cardiovascular disease is the main cause of mortality in patients with diabetes, and risk reduction is an important goal in the treatment of diabetes. The UKPDS study demonstrates that the antihyperglycemic drug metformin is associated with a reduction in cardiovascular events and death compared to conventional treatment. AMP-activated protein kinase (AMPK) is an important enzyme concerning glucose and lipid metabolism, which has recently been found to play an important protective role in the ischemic heart. Previous studies have provided evidence that some of the beneficial effects of metformin in liver and skeletal muscle might be through activation of AMPK. The aim of this study was to determine whether a single dose of metformin was capable of protecting the myocardium against experimentally induced ischemia 24 hours after the intervention, and if so to assess whether the AMPK system might be involved.
Materials and Methods: Wistar rats (~ 300 g) were allocated into two groups: a metformin group given a single dose (oral gavage) of metformin (250 mg/kg body weight), and a control group given a single oral dose of vehicle (NaCl). After 24 hours the hearts were perfused in a Langendorff model and subjected to 45 minutes left main coronary artery occlusion followed by 120 minutes reperfusion. Infarct size was determined by tetrazolium staining and expressed as a percentage of the risk zone (I/R%). Isoform specific AMPK-alpha2 activity was measured 2 hours after administration of metformin or vehicle.
Results: Infarct size was significantly reduced in the metformin treated (I/R: 20 ± 4% vs. 38 ± 4%, $p < 0.01$, $n = 8$) compared to the control group. A single oral dose of metformin resulted in an approximately 2-fold increase in AMPK-alpha2 activity ($p < 0.015$, $n = 4–8$).
Conclusion: A single dose (oral gavage) of metformin significantly increases AMPK-activity 2 hours after administration and reduces the infarct size seen after a coronary artery occlusion 24 hours after administration. Increased AMPK-activity may be an important signal mediator involved in the mechanisms behind delayed cardioprotection afforded by metformin.

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Ectopic lipid accumulation in myocardium related with increased RAAS activities and cardiac dysfunction
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Background and Aims: Obesity, especially when accompanied by type 2 diabetes, can lead to cardiac dysfunction. It is known that lipid accumulation is associated with cardiac dysfunction in obesity and recent studies revealed that RAAS (especially in myocardium) plays an important role in myocardial hypertrophy and myocardial dysfunction of obese people. The present study investigated the relationship between heart lipid accumulation and RAAS activities in obese S-D rat and examined whether these changes are correlated with myocardial dysfunction.
Materials and Methods: Two groups of S-D rat fed high-fat diet (OB) or standard laboratory chow(C) respectively for 24 weeks. Whole-body insulin sensitivity was assessed by a hyperinsulinemic- euglycemic clampat the end of 24th week. Then the maximum velocity of myocardial contraction (+dP/dt max) and the maximum velocity of myocardial diastole (–dP/dt max) of intracardiac pressure were measured by physiological polygraph. Blood samples for triglyceride (TG), free fatty acids (FFAs) and angiotension II (Ang II) measurements were collected. Rats were killed and the ventricular portion of the heart was immediately placed in liquidN2 and stored until TG, FFAs and AT II analysis.

Results:The insulin infusion rates during clamp were much lower in OB group (OB:15.16 ± 1.75 mg/kg*min vs. C:23.01 ± 1.98 mg/kg*min, P<0.05). The concentrations of TG and FFAs in the blood and myocardium were significantly elevated in OB group (P<0.05) when compared with controls and Ang II concentration, either in myocardial homogenate or blood (P<0.05), were increased (table 1) in OB group. The values for +dP/dt (max) and –dP/dt (max) were lower in OB group than that in C group (+dP/dt max: OB: 1073.3 ± 211.0 mmHg/s, C:1489.7 ± 214.3 mmHg/s, P<0.05; –dp/dt max: OB: –1068.1 ± 305.4 mmHg/s, C:–1508.5 ± 208.9 mmHg/s, P<0.05). Blood Ang II level was correlated with the concentration of TG(r=0.839, P<0.001) and FFAs (r=0.942, P<0.001, table 1)). Myocardial Ang II concentrations was correlated with TG (r=0.766) and FFAs (r=0.802) (P<0.01). The values of +dp/dt (max) and –dp/dt (max) were correlated with the concentrations of TG(r=–0.561 and r=–0.609), FFA(r=–0.557 and r=–0.544) and Ang II (r=–0.746 and r=–0.595) in myocardium (P<0.05).

Table 1. Concentrations of TG, FFAs and Ang II in blood and myocardium

		TG (mmol/l)	FFAs (umol/l)	AngII (pg/ml)
NC	blood	2.03 ± 0.55	386.9 ± 91.4	145.4 ± 21.2
	myocardium	1.89 ± 0.32	251.6 ± 44.3	88.0 ± 8.8
Ob	blood	4.63 ± 0.78	834.9 ± 89.1	360.8 ± 56.5
	myocardium	2.96 ± 0.51	398.9 ± 64.2	152.5 ± 12.8

Conclusion: In insulin resistance state ectopic lipid accumulation in myocardium as the results of elevated circulating FFAs and TG concentration impairs cardiac systolic and diastolic functions. Since myocardial Ang II levels in obese rats was positively correlated with TG and FFAs concentration, and the cardiac function was negatively correlated with the TG, FFAs and Ang II levels in myocardium, it is logical to deduce that ectopic lipid accumulation in myocardium may increase RAAS activities, both of them have a important role to increased risk of congestive heart failure in obese subjects.

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Insulin modulates cardiohaemodynamic disturbances induced by acute myocardial ischemia

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Background: It was recently shown that hyperinsulinemia could directly take part in pathogenesis of cardiovascular deterioration, especially in presence of endothelial dysfunction. On the other hand, we propose that hyperinsulinemia, for example in patients with type 2 diabetes mellitus, can change sensitivity of heart to acute myocardial ischemia (AMI) by insulin promoting similar signaling pathways deteriorations.
Aims: We have sought to examine this problem, namely quantify and qualify insulin-induced modulation of cardiohaemodynamic reactions to AMI.
Materials and Methods: Experiments on 26 healthy dogs under chloralose anesthesia (30–100 mg/kg, i.v.) were performed. Catheterization, extracorporeal programmed autoperfusion of circumflex coronary artery (CA) with constant volume of arterial blood, heart and main vessels catheterization, catheterization and continuous drainage of coronary sinus were carried out. Left and right ventricular pressures, maximal velocity of left ventricular pressure elevation ($+dP/dt_{max}$) and reduction ($-dP/dt_{max}$), coronary sinus blood oxygen saturation, arterial blood pressure, CA and femoral artery resistances were registered.
Results: In model experiments with AMI induced by interruption of CA perfusion (60 s) we observed significant diminution of heart contractility (decrease of $+dP/dt_{max}$ from 385 ± 45 to 340 ± 38 kPa/s and $-dP/dt_{max}$ from 370 ± 38 to 335 ± 32 kPa/s), femoral artery perfusion pressure (from 16.5 ± 3.3 to 13.5 ± 2.0 kPa), arterial blood pressure (from 15.4 ± 2.5 to 8.2 ± 1.9 kPa), coronary sinus blood oxygen saturation (from 33.1 ± 6.1 to 28.4 ± 5.2%), left and right ventricular systolic pressures (from 19.5 ± 3.9 to 15.0 ± 2.3 kPa and from 6.8 ± 1.2 to 4.1 ± 1.0 kPa, resp.). CA resistance in process of reperfusion, determined by magnitude of the degree of CA perfusion pressure, was dramatically decreased (17.2 ± 3.2 kPa – in control and 13.0 ± 2.3 kPa – in a reperfusion state). On the 30th minute after insulin injection (1.0 IU/kg, i.v.) AMI was followed by less pronounced decrease of heart contractility (descent of $+dP/dt_{max}$ from 352 ± 63 to 335 ± 58 kPa/s and $-dP/dt_{max}$ from 346 ± 61 to 330 ± 55 kPa/s), femoral artery perfusion pressure (from 14.3 ± 2.1 to 12.7 ± 1.4 kPa), arterial blood pressure (from 14.0 ± 1.4 to 12.3 ± 1.5 kPa), left and right ventricular systolic pressures (from 16.7 ± 3.2 to 14.5 ± 2.8 kPa and from 5.4 ± 1.1 to 4.8 ± 0.9 kPa, resp.) and increase of CA reperfusion pressure (15.1 ± 3.1 – in control and 13.8 ± 2.2 – in a reperfusion state). But the degree of coronary sinus blood oxygen saturation descent, induced by AMI, on the contrary, was significantly increased (from 37.2 ± 6.0 to 27.1 ± 5.4%). ECG deteriorations during AMI were also augmented. The similar effects of AMI on cardiohaemodynamic were also observed after M-cholinergic receptors blockade (atropine 0.5 mg/kg, i.v.).
Conclusion: These data demonstrate that hyperinsulinemia can provoke more pronounced damage of heart metabolism and function induced by AMI and it does not correlate with the degree of cardiohaemodynamic parameters changes.

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Cardiac function and mass in asymptomatic type 1 diabetic patients with diabetic nephropathy in relation to NT-proBNP - a cardiovascular magnetic resonance study

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Background and Aim: The increased cardiovascular mortality in type 1 diabetics is predominantly caused by a poor prognosis in patients with diabetic nephropathy. Cardiovascular magnetic resonance imaging (CMR) is the gold standard for evaluation of left ventricular function (LVF) and anatomy. We sought to evaluate left ventricular mass (LVM), volumes and function in asymptomatic type 1 diabetic patients with and without diabetic nephropathy. Furthermore, to investigate if CMR measures correlated to N-terminal pro-brain natriuretic peptide (NT-proBNP), an independent predictor of cardiovascular mortality and morbidity in patients with diabetic nephropathy.
Methods: In a case-control study, 123 patients with type 1 diabetes without symptoms or history of cardiac disease were examined. We included 60 patients with diabetic nephropathy (34 men, age (mean (SD)) 49 ± 10 years, diabetes duration 33 ± 9 years, BMI 24 ± 3 kg/m^2, HbA_{1c} 8.8 ± 1.1%, blood pressure 137 ± 19/74 ± 9 mmHg, urinary albumin excretion rate (UAE) (median (range)) 352 (3–2632) mg/24 h, and Glomerular filtration rate (GFR) 67 ± 34 ml/min/1.73 m^2) and a control group of 63 patients with persistent normoalbuminuria (37 men, age 52 ± 9 years, diabetes duration 31 ± 7 years, BMI 25 ± 3 kg/m^2, HbA_{1c} 8.0 ± 1.0%, blood pressure 131 ± 19/72 ± 11 mmHg, and UAE 8 (2–29) mg/24 h. Diabetic nephropathy was defined as previously persistent UAE > 300 mg/24 h. Global LVF, LVM, volumes and diastolic function was evaluated by CMR (1.5 Tesla Philips-NT MR scanner). NT-proBNP was determined with an immunoassay.
Results: All patients had normal global LVF. LVM and NT-proBNP values were significantly increased in patients with diabetic nephropathy (table). Patients with nephropathy had smaller left ventricular volumes and a higher E/E' ratio suggestive of diastolic dysfunction with increased left ventricular filling pressures.

MR parameters and NT-proBNP in 123 type 1 diabetic patients with and without diabetic nephropathy

	Patients with normoalbuminuria (n=63)	Patients with nephropathy (n=60)	p value
LVM adjusted for BSA (g/m^2)	47.1 ± 8.8	56.3 ± 14.0	**<0.0001**
Ejection fraction %	68 ± 8	69 ± 9	0.4
Cardiac output (l/min)	6.3 ± 1.4	6.3 ± 1.4	0.9
Heart rate (beats/min)	75 ± 11	82 ± 11	**0.001**
E/E' ratio	3.7 ± 1.5	5.2 ± 3	**0.03**
LVEDV (ml)	126.0 ± 30.7	110.7 ± 26.6	**0.004**
LVESV (ml)	41.6 ± 17.6	35.4 ± 15.1	**0.04**
NT-proBNP (ng/l)	44(6–621)	72(5–3718)	**0.03**

Data are means±SD, or medians (range).
LVM = left ventricle mass.
BSA = Body Surface Area((height in cm × weight in kg)3600)$^{1/2}$

LVEDV = Left ventricular end diastolic volume
LVESV = left ventricular end systolic volume
E/E' ratio = peak mitral inflow velocity (E) divided by peak mitral annulus velocity (E')

Of the CMR measures in patients with diabetic nephropathy only LVM was correlated to NT-proBNP ($r = 0.46$, $p<0.0001$) in univariate analysis. Furthermore, NT-proBNP was positively correlated to blood pressure and age; and negatively correlated to GFR, BMI, and haemoglobin.
Conclusion: CMR demonstrated increased LVM and filling pressures in asymptomatic type 1 diabetic patients with nephropathy compared to normoalbuminuric patients. In patients with diabetic nephropathy increased LVM contribute to elevation in NT-proBNP – both markers of increased cardiovascular risk in diabetic patients with elevated UAE.
Support: Roche Diagnostics

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Cardiac stent-induced activation of monocytes in type 1 diabetes
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Background and Aims: Angioplasty is the surgical procedure of choice to eliminate obstructions in atherosclerotic coronary vessels. However, balloon expansion at the site of stenosis damages the vessel wall. Consequent tissue repair often leads to the formation of an excess of neointimal tissue leading to a second obstruction (restenosis). Deployment of a metal stent offers a mechanical scaffold and reduces the incidence of restenosis. However, the contact of the stent with the regenerating tissue triggers a host response leading to high levels of inflammatory cells in the first 30 days following implantation. Inflammatory cells are activated by contact with stent stainless steel surface, leading to production of growth factors (TGFβ, PDGF) inducing smooth muscle cell proliferation and formation of neointima (in-stent restenosis). Incidence of in-stent restenosis is increased in diabetic subjects.The present work examines the effect of stainless steel (St) on the activation of inflammatory cells in Type 1 diabetes patients and the potential role of their inflammatory cells in the development of in-stent restenosis.
Materials and Methods: Human mononuclear cells (HM) were gradient isolated from the human peripheral blood of 14 control and 14 Type 1 diabetes subjects. To study HM adhesion, cells were incubated (3 h) onto stainless steel surfaces and tissue culture plates (TCP). Adhering cells were analysed by scanning electron microscopy (SEM). Cell supernatants were either assayed for Interleukin 1β (IL-1 β), tumour necrosis factor α (TNF α) and transforming growth factor β (TGFβ) using ELISA or analysed for the presence of platelet-derived growth factor (PDGF) by Western blotting.
Results: SEM showed that HM from both groups adhered to St with a relatively high degree of spreading and with a rough plasmalemma, suggesting their activation. Cytokine evaluation highlighted a well-defined pattern of St-inducedactivation. Pro-inflammatory cytokines such as TNFα and IL-1β were secreted by the cells in contact with TCP and St only at very low levels. Conversely, TGFβ secretion on cells adhering to St was at levels significantly higher than TCP in both control and Type 1 diabetes subjects (Table 1). Western blot for PDGF showed that St-incubated control and Type 1 diabetes cells secreted significant levels of this potent growth factor. No significant difference between the two groups was found.

Table I: St effect on HM TGFβ secretion.

Sample	Control*	Type 1*
TCP	408.5 ± 127.1	331.5 ± 95.1
St	**612.3±117.5 †**	**535.2±117.3 ††**

*Data are expressed as pg/ml±standard error
†indicates values significantly different from TCP at $p<0.05$.
††indicates values significantly different from TCP at $p<0.01$.

Conclusion: The results of the present work confirms that when in contact with stainless steel, HM secrete potent activators of SMC proliferation such as TGFβ and PDGF rather than pro-inflammatory cytokines thus enhancing the risk of restenosis. However, the higher incidence of restenosis in diabetic patients seems to be independent of the inflammatory response since these subjects showed comparable levels of HM activation to the controls.
Support: NHS R&D grant

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Effects of the omega-3 polyunsaturated fatty acids in the treatment of cardiovascular autonomic neuropathy in type 2 diabetes mellitus patients
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Background and Aims: Dietary supplementation with fish oil, a source of highly long chain marine polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has been proposed as an antithrombotic and antiatherosclerotic therapy. The aim of this study was to assess the long term effect (4 months) of DHA and EPA on the heart rate variability (HRV), Ewing's battery tests, QTc interval parameters, the activities of membrane-bound enzymes in the membranes of erythrocytes (RBC's), state of the prostacyclin I_2-thromboxane A_2 system, parameters of the platelet aggregation in patients with Type 2 diabetes mellitus (Type 2 DM) and cardiovascular aunonomic neuropathy (CAN).
Materials and Methods: CAN was established by reduced HRV, Ewing's battery tests and QTc interval parameters disturbances. 39 pts with CAN (54 ± 5 years, 22 m/17f) were allocated in two treatment groups. Pts of group A (n=26) were receiving capsules of fish oil every day (2,0 g EPA, 2,0 g DHA and 0,1% α-tocopherol acetate) and the group B (n=13) was receiving placebo capsules of olive oil. All patients were on the same diet. The duration of the study was 4 months. We investigated the following parameters: the activities of protein-kinase C (PK-C), Na^+, K^+-ATPase, Ca^{2+}, Mg^{2+}-ATPase, fatty acids composition in the membranes of RBC's, erythrocytes, levels of the ^{125}I-6-ketoprostaglandin $F_{1\alpha}$ (6-ketoPGF$_{1\alpha}$) and ^{125}I-thromboxane B_2 (TXB_2) in the blood plasma. Platelets were exposed to different agonists (1 U/ml of thrombin; 0,1; 0,5 and 5 μM of ADP). ADP-induced platelet aggregation was measured by automatic system. Statitics: one way analysis of variance (ANOVA).
Results: It has been discovered that manifestation of the CAN is accompanied by decrease of the Na^+, K^+-ATPase, Ca^{2+}, Mg^{2+}-ATPase activities ($p<0,001$), 6-ketoPGF$_{1\alpha}$, EPA level ($p<0,001$), EPA/arachidonic acid ratio with simultaneous increase of TXB_2 level, PK-C activity, QTc interval parameters. Analysis of aggregatory curves shows that platelets in Type 2 DM with CAN begin to aggregate earlier and the speed (0,79 ± 0,03 U/min, $p<0,001$), stage of aggregation (29,37 ± 1,18 MU/min, $p<0,01$) increase. Obtained results could witness about increase of platelet sensitivity towards thrombin and ADP. After 4 months of treatment there were a decrease of TXB_2 level (141,2 ± 15,4 pg/ml, $p<0,001$), activity of PK-C (14,46 ± 4,52 pmol ^{32}P/mg protein per 1 min, $p<0,001$), degree and speed of an aggregate of thrombocytes with simultaneous increasing of EPA level, EPA/arachidonic acid ratio, activities of Na^+, K^+-ATPase (from 0,04 ± 0,003 to 0,1 ± 0,004 mMol P_i/mg protein per 1 hour, $p<0,001$), Ca^{2+}, Mg^{2+}-ATPase and the level of the 6-ketoPGF$_{1\alpha}$ in the group A marked. Also, we observed significant improvement of cardiovascular autonomic tests, HRV parameters, decrease of QTc interval ($p<0,01$). Increase of the of EPA level, EPA/arachidonic acid ratio and the activities of membrane-bound enzymes lead to the increase of RBC's deformability. Therefore it seems that a 4,0 g fish oil treatment during following 4 months result in the tendency of normalizing the state of prostacyclin I_2-thromboxane A_2 system, activity of membrane-bound enzymes, HRV, QTc parameters and cardiovascular autonomic tests.
Conclusions: In conclusion, DHA and EPA at moderate doses may exert antithrombotic effects and may be used for effective prophylaxis and treatment of diabetic cardiovascular autonomic neuropathy.

OP 4

Insulin action in human muscle

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The impaired capacity to switch between fat and glucose oxidation in skeletal muscle of impaired glucose tolerant men during fasting and after a meal improves after weight loss

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Background and aims: Diabetes mellitus type 2 and insulin resistance are associated with an accumulation of triglycerides in skeletal muscle, possibly due to a dysbalance between free fatty acid (FFA) uptake and oxidation. The aim of this study was to investigate: 1) whether FFA uptake and oxidation were disturbed during fasting and after a high fat meal in skeletal muscle of obese men with impaired glucose tolerance (IGT), a prediabetic state, compared to obese men with normal glucose tolerance (NGT); 2) whether the observed disturbances were accompanied by changes in intramyocellular triglycerides (IMTG) and; 3) whether the disturbances in IGT men improved after weight loss.
Material and methods: Substrate fluxes over skeletal muscle were calculated with arterial and deep venous concentrations and blood flow over forearm muscle during fasting and after a high fat meal (61 en% fat, 33 en% carbohydrates). Muscle respiratory quotient (RQ), lipid and carbohydrate oxidation were calculated from O_2 and CO_2 flux. Forearm muscle FFA uptake was measured using the tracer [2-^{2}H]-palmitate. IMTG was measured with ^{1}H-magnetic resonance spectroscopy in *m. vastus lateralis*. Measurements were repeated in IGT men after a 12-week weight loss period (4 wk VLCD, 4 wk low calorie diet, 4 wk energy balance, –15% of body weight).
Results: Insulin sensitivity, as measured during clamp, tended to be lower ($p = 0.09$) in IGT ($n = 11$) than in NGT men ($n = 9$) and improved after weight loss (WL) in IGT men ($n = 8$, $p = 0.001$). After the meal, arterial glucose and insulin tended to be higher ($p = 0.06$, $p = 0.07$ resp.) in IGT men. WL reduced postprandial hyperglycemia (–8%, $p = 0.02$) and hyperinsulinemia (–40%, $p = 0.01$). Forearm muscle FFA uptake during fasting and after the meal was comparable between IGT and NGT, and in IGT before and after WL. Forearm muscle RQ was not different in IGT and NGT during fasting, but increased more in NGT after the meal (incremental area under the curve (iAUC) in NGT = 0.053 ± 0.087, iAUC in IGT = -0.033 ± 0.050, $p = 0.03$), indicating an impaired suppression of muscle fat oxidation in IGT. After WL in IGT men, fasting muscle RQ was reduced ($p = 0.03$), reflecting an improved fat oxidation ($p = 0.02$), and increased more after a meal (iAUC before WL = -0.033 ± 0.050, iAUC after WL = $+ 0.039 \pm 0.105$, $p = 0.03$), reflecting trends for an improved suppression of fat oxidation ($p = 0.09$) and an improved stimulation of carbohydrate oxidation ($p = 0.06$). This was accompanied by a reduction in IMTG (–15%, $p = 0.08$), which correlated strongly with fat mass loss (Pearson Corr. Coef. = 0.79, $p = 0.03$).
Conclusions: Skeletal muscle of obese IGT men showed an impaired capacity to switch from fat to carbohydrate oxidation in the postprandial state as compared to obese NGT men, demonstrating metabolic inflexibility of substrate oxidation. As obese IGT subjects are at high risk of developing DM2, this indicates that impaired metabolic flexibility may play a role in the early stages of development of DM2. As the disturbed metabolic flexibility improves markedly after weight loss, this indicates that this disturbance is for a large part secondary to the obese, insulin resistant and/or glucose intolerant state.
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Upstream insulin signalling in non-diabetic twins – the impact of heredity, age, zygosity and birth weight and the relation to insulin-stimulated in vivo glucose and fat metabolism

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Background and Aims: Insulin resistance in skeletal muscle tissue represents a major defect in type 2 diabetes. The aim of this study was to evaluate the relative impact of genetic versus non-genetic factors on the activities and protein levels of key upstream insulin signalling molecules (protein levels of insulin receptor (IR), insulin receptor substrate 1 (IRS1) and the regulatory subunit (p85) of phosphoinositide 3-kinase (PI3K) as well as the activities of IR tyrosine kinase and IRS1 associated PI3K) and to assess their relation to measures of in vivo glucose metabolism in young and elderly monozygotic (MZ) and dizygotic (DZ) twins.
Materials and Methods: A total of 184 twins (young MZ: n=63; DZ: n=38; elderly MZ: n=40; DZ: n=43) underwent a 2-hour euglycaemic, hyperinsulinaemic clamp (40 mU/m^2/min) combined with indirect calorimetry and excision of muscle biopsies during basal and insulin stimulated states. Biometric modelling was applied to the data in order to estimate components of variance (additive genetic a^2, shared c^2 and unique environment e^2). Multiple regression analyses were performed to investigate the relation between signalling proteins and in vivo metabolism.
Results: Biometric modelling revealed a genetic component in the IRS1-PI3K activity during the basal state in young twins ($a^2 = 35\%$) and during insulin-stimulation within elderly twins ($a^2 = 83\%$). Furthermore, insulin-stimulated IRTK activity was under predominant genetic control in elderly twins ($a^2 = 65\%$), whereas there was no genetic component among younger twins. IR, IRS1 and p85 proteins were exclusively under environmental control in both age groups. In the regression analyses age, sex, zygosity, birth weight, BMI, VO_2 max, total, trunk and leg fat percentages were included as explanatory variables and IR, p85 and IRS1 proteins, IRTK and IRS1-PI3K activities as response variables, respectively. IR protein was influenced by sex, VO_2max and total fat %. p85 protein was associated to zygosity, sex, total fat % and VO_2max. IRS1 protein was associated with zygosity, birth weight and the interaction age x zygosity. IRTK activity was influenced by age whereas IRS1-PI3K activity was not associated to any of the included variables. When glucose disposal (Rd), non-oxidative glucose metabolism (NOGM), glucose (GOX)and fat oxidation (FOX) were set as response variables, Rd was not associated with any activities or protein levels of the signalling molecules. NOGM was negatively associated to IRS-1 protein, GOX was positively associated to p85 and IR proteins and FOX was associated to IR protein in a negative and to IRTK activity in a positive way.
Conclusion: We demonstrate a major genetic component in the control of IRS1-PI3K activity in both young and elderly twins, and on IRTK activity among elderly twins. The IR, p85 and IRS1 protein levels were exclusively under non-genetic control. The fetal environment expressed as zygosity and/or birth weight had significant influences on p85 and IRS1 proteins. Interestingly, insulin stimulated Rd was not associated with any of upstream protein signalling levels or activities. However, GOX and FOX were both influenced by IR protein as well as p85 protein and IRTK activity, respectively.

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Investigation of the effects of calpain-10 inhibition on insulin action in cultured skeletal muscle cells

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Background and Aims: Variation within the calpain-10 (CAPN10) gene, a putative type 2 diabetes susceptibility gene, has been associated with an increased risk of developing type 2 diabetes. However, the physiological function of calpain-10 remains poorly understood. Calpain-10 is expressed in many insulin-responsive tissues, particularly skeletal muscle, liver and the pancreas. A number of studies have indicated that calpain-10 may have an influence on insulin secretion and insulin action. It has also been suggested that, in some populations, decreased expression of CAPN10 may be associated with insulin resistance in skeletal muscle. The aim of this study is to investigate the effect of calpain-10 inhibition on insulin action in cultured skeletal muscle cells.
Materials and Methods: Cultured myoblasts were transfected with short interfering RNAs (siRNAs) specific for calpain-10 in order to inhibit expression of this gene. Two days after transfection, cells were induced to differentiate. All studies described were performed on transfected day 7 myotubes. Quantitative PCR and Western blotting were employed to assess the extent of inhibition of calpain-10 expression compared with untransfected cells. Insulin-stimulated glucose uptake and glycogen synthesis was measured in these transiently transfected cells to assess the effects of calpain-10 inhibition on insulin action.
Results: Transfection of cultured skeletal muscle cells with siRNAs specific for calpain-10 resulted in specific inhibition in the expression of calpain-10. Analysis of expression by quantitative PCR showed that expression of CAPN10 was reduced by 83% in transfected cells compared to untransfected and mock transfected cells. Cells were also transfected with a negative control to assess the effects of introducing siRNAs into these cells. This reduction in gene expression was also seen at the protein level as confirmed by Western blotting with an antibody specific for calpain-10. Insulin-stim-

ulated glucose uptake and glycogen synthesis were then measured in response to calpain-10 inhibition. While untransfected, mock transfected and cells transfected with a negative control gave a comparable increase in glucose uptake in response to 1 μM insulin (fold increase above basal; 1.434 ± 0.16, 1.403 ± 0.12 and 1.495 ± 0.23, respectively, mean ± SEM), cells expressing reduced levels of calpain-10 showed a decreased response to insulin (1.03 ± 0.06). In contrast, there were no marked differences in insulin-stimulated glycogen synthesis in response to calpain-10 inhibition.
Conclusion: These results show that calpain-10 expression has been specifically inhibited in cultured skeletal muscle cells. Inhibition of calpain-10 expression results in a reduced insulin response to glucose uptake suggesting that calpain-10 may play a role in insulin-stimulated glucose uptake in skeletal muscle cells.
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Skeletal muscle ceramide content in lean, obese and glucose intolerant subjects
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Background and Aims: There are data that intramuscular lipids might be responsible for the development of insulin resistance. We recently demonstrated that insulin sensitivity is inversely related to muscle content of ceramide, a second messenger in the sphingomyelin signaling pathway. The aim of the present study was to assess muscle ceramide and sphingomyelin content and composition of fatty acids and to evaluate their relationships with insulin sensitivity in lean, obese and glucose intolerant subjects.
Materials and Methods: The study group consisted of 10 lean (BMI <25 kg × m^{-2}) healthy male subjects (control group) and 18 male subjects with overweight or obesity – 10 with normal glucose tolerance (obese-NGT) and 8 with impaired glucose tolerance (obese-IGT). Euglycemic hyperinsulinemic clamp and a biopsy of vastus lateralis muscle were performed. To avoid contamination of extracellular fat, muscles were lyophilized. Ceramides and sphingomyelins were separated with thin-layer chromatography. The content of particular FA was determined by gas-liquid chromatography. Activities of neutral and acid sphingomyelinases and content of sphinganine (intermediate in de novo ceramide synthesis) and sphingosine (product of ceramide hydrolysis) in muscle were also measured.
Results: Both groups of obese subjects were markedly more insulin resistant in comparison to controls (obese-NGT, p=0.008 and obese-IGT, p=0.00053) and obese-IGT group had also lower insulin sensitivity than obese-NGT (p=0.012). Muscle ceramide content was higher in both groups of obese subjects in comparison to controls (obese-NGT, p=0.034 and obese-IGT, p=0.00038) and in obese-IGT in comparison to obese-NGT group (p=0.0034). Also, both groups of obese subjects demonstrated higher muscle sphinganine content than controls (obese-NGT, p=0.049 and obese-IGT, p=0.0034). Muscle ceramide and sphinganine were inversely related to insulin sensitivity (r= –0.57, p=0.0014 and r= –0.48, p=0.01, respectively). Muscle sphingomyelin content was lower in obese-IGT than in obese-NGT group (p=0.0076). Additionally, obese-IGT subjects had an increased muscle neutral sphingomyelinase activity in comparison to the remaining groups (both p=0.001).
Conclusion: Our data show that muscle ceramide accumulation might be responsible for the development of insulin resistance in subjects at risk of developing type 2 diabetes. In obese-NGT subjects, ceramide accumulation is probably the result of an increased de novo synthesis, as indicated by sphinganine content. In obese-IGT individuals, sphingomyelin hydrolysis due to an increased neutral sphingomyelinase activity also seems to be an important factor.
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Triacylglycerol accumulation is not primarily affected in myotubes established from type 2 diabetic subjects
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Background and Aims: The aim of this study is to clarify to which extent triacylglycerol (TAG) accumulation in skeletal muscle *in vivo* depends on the genetic background or on environmental factors i.e. depends on chronic exposure to free fatty acid (FFA), insulin, and glucose, and whether this may be followed by induction of insulin resistance. To distinguish environmental from genetic factors, we choose to study human myotubes from healthy, lean, and obese subjects with and without type 2 diabetic (T2D).
Materials and Methods: Human myotubes established from 10 lean, 10 obese, and 10 T2D subjects were allowed to differentiate for eight days. The last four days, myotubes were exposed to increasing palmitate (PA) and oleate (OA) concentrations with/without high glucose and/or high insulin concentrations. Subsequently, we studied TAG accumulation, glucose- and fatty acid (FA) uptake and glycogen synthesis (GS) under acute insulin stimulation and basal conditions.
Results: We found an increased TAG accumulation in myotubes exposed to chronically increased PA and OA concentrations established from all three groups ($p < 0.001$) with no differences between groups ($p > 0.05$). Chronic high insulin, but not high glucose, concentrations increase TAG accumulation by 25% ($p<0.001$). Basal and insulin-stimulated FA uptake showed a significantly positive correlation to the TAG content after chronic exposure to increasing PA and OA concentrations ($p < 0.001$). Both chronic PA and OA exposure reduced the insulin-mediated PA and OA uptake (fold change) ($p < 0.001$), but could not induce insulin resistance at the level of glucose uptake in the three myotube groups, whereas high insulin concentrations induced insulin resistance ($p < 0.001$). Chronic high PA, but not OA, induced insulin resistance at the level of GS in myotubes from control subjects ($p < 0.05$). TAG content correlated negatively with insulin-mediated FA uptake ($p < 0.001$), but did not correlate with insulin-stimulated glucose uptake for PA or OA ($p > 0.05$).
Conclusion: These results indicate that 1) TAG accumulation is not primarily affected in obese and T2D; 2) chronically increased FA levels and hyperinsulinemia may be followed by increased TAG accumulation intramuscularly *in vivo*; and 3) increased TAG accumulation in diabetic subjects may depend on increased FA and insulin load and may be enhanced by increased incorporation of FA into TAG with increasing TAG. Both saturated and unsaturated FA can induce insulin resistance at the level of lipid uptake in skeletal muscle. However, only saturated FAs can induce insulin resistance at the GS level. TAG is not a mediator of insulin resistance.
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Effect of chromium picolinate on AMP-activated protein kinase α subunits and acetyl coenzyme A carboxylase in primary cultured human skeletal muscle cells
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Background and Aims: Chromium Picolinate (CrPic) has been suggested as a cofactor for insulin action. Data has been obtained that has suggested that CrPic may enhance cellular signaling through the insulin receptor and increase GLUT-4 translocation. However, the precise mechanism by which chromium improves glucose metabolism and enhances insulin sensitivity is still poorly understood. One pathway that has not been evaluated adequately with CrPic is the AMP-activated protein kinase cascade. The AMP-activated protein kinase cascade, in addition to maintaining the energy status of individual cells, plays a pivotal role in whole body energy balance and in development of insulin resistance. AMPK when activated increases the AMP/ATP ratio within the cell in response to metabolic stresses. It is well established that exercise, AICAR, adiponectin, and leptin can stimulate AMPK activity. However, the effect of chromium on AMPK activity in humans is unknown. To test the effect of CrPic on the AMPK system, we measured the activity of AMPK α1, α2, and analyzed the abundance of AMPK α1, α2 and acetyl coenzyme A carboxylase (ACC) protein levels in primary human skeletal muscle culture. We also analyzed mRNA expression in the primary cultured human skeletal muscle cells pretreated with chromium picolinate (CrPicc).
Materials and Methods: Primary cell culture was initiated from skeletal muscle biopsies and grown to approx. 80% confluence. Cultures were then incubated in the presence of CrPic (2.5, 5, 10, 20 or 50 ng/ml) or control media only. Total RNA was isolated after 16 hours of treatment. ACC, AMP a1 and α2 levels and phosphorylation were assessed with Western Blot techniques after immunoprecipitation. In addition, specific activities for AMPK were assessed. Gene expression for AMPK α1 or α2 was assessed by real-time PCR assays.
Results: CrPic was shown to significantly increase phosphorylation (Thr^{172}) of AMPK α1 and α2 after immunoprecipitation with anti-AMPK α1 or α2 antibody, respectively. ACC-phosphorylation was also increased with CrPic. In addition, CrPic treatment significantly increased AMPK α1 and α2 activities in a concentration and incubation time dependant manner. However, it was observed that CrPic selectively elevated AMPK α2 protein abundance, but decreased AMPK α1 protein levels with a CrPic concentration increase.

The changes in protein content were confirmed by the real time RT-PCR assay.
Conclusion: This study suggests that CrPic may selectively increase AMPK α2 abundance, but inhibit AMPK α1 protein expression at higher concentrations. CrPic could directly stimulate phosphorylation of both AMPK α1 and AMPK α2 sub-units as an allosteric activator followed by increasing phosphorylation of ACC. These effects of CrPic on AMPK alpha isoforms and ACC may be one of the mechanisms of CrPic improving glucose and lipid metabolism.

OP 5

Beta cell neogenesis and differentiation

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Engineering of multipotent human stem cells from pancreatic islets of Langerhans
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Background: The aim of the study was to isolate, immortalize and characterize a pure population of nestin expressing cells from human islets of Langerhans by the strategy of isolation of single cell derived colonies combined with a promoter targeted selection of nestin expressing cells.
Objective and approach: Highly purified human islets were cultured in RPMI 1640 medium (with 10% FCS), and enriched for nestin expressing cells by addition of the mitogens bFGF and EGF (20 ng/ml). The proliferating cells were then subjected to a FACS procedure that allows the transfer of single cells into a 96-well tissue culture dish. After 10 to 14 days we observed the formation of proliferating colonies in 2.6% of the wells (of total 192). Using RT-PCR a strong nestin mRNA expression but no insulin expression was found in all colonies. In order to ensure stable proliferation rates, cells from best growing clone BC11 were reversibly immortalized using Lox-CRE excisable lentiviral vectors expressing the hTelomerase and mBmi-1, a combination of genes described to be optimal for reversible immortalization of primary human cells (Salmon et al., Mol Ther 2000). The immortalized cells BC11-BT were then transduced by a lentivector containing a transcription cassette expressing GFP under the control of nestin promoter with a neural specific enhancer element from the 2nd intron (nestGFP).
Results: At any given time (up to passage 75) the reversibly immortalized and nestGFP-transduced cells continued to express nestin. Besides nestin, these cells also express other stem cell markers including the side population marker ABCG2, the hepatic stem cell marker Thy-1, the stem cell factor and the stem cell factor receptor. In addition, they express Isl-1, a transcription factor that is crucial for the development of pancreatic endocrine cells. Using a monoclonal Isl-1 antibody we found positive nuclear staining in almost all cultured cells. Upon differentiation in serum-free DMEM/F12 medium, the cells expressed insulin and the transcription factors Nkx2.2 and Pax4. Immunocytochemistry and electron microscopy studies also indicated the presence of C-peptide positive cells. In another set of experiments these cells showed the capability to differentiate into adipocytes with formation of intracellular fat and expression of adipocyte-specific genes. Culturing in an osteogenic medium resulted in an osteoblast-like phenotype with induction of alkaline phosphatase and mineralization. Furthermore, the immortalized cells expressed human albumin in vivo after transplantation into SCID mouse liver.
Conclusions: We have successfully immortalized cells from human islets that are multipotent as shown by their differentiation in vitro into insulin expressing cells, adipocytes and osteoblast-like cells, as well as in vivo into human albumin producing hepatocytes. The combination of different stem cell markers together with Isl-1 indicates that these cells have the features of mesenchymal pancreatic stem cells.

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In vitro neogenesis of human islets reflects the plasticity of differentiated human pancreatic cells
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Background and Aims: Neogenesis of islets from cultured human adult pancreatic tissue has been reported. The islet progenitors have been thought to represent ductal cells. However, recent transgenic mouse experiments have proposed that replication of pre-existing beta cells is the predominant mechanism operating in the adult animal. Since previous experiments have been "contaminated" by a number of pre-existing islet cells, we studied their involvement in the islet neogenesis.

Materials and Methods: Fresh isolated human pancreatic cells with different purity of islets were expanded in monolayer and induced to differentiate as described by us previously (Gao et al. Diabetes 52:2007–2015, 2003). Endocrine cell proliferation was assessed by BrdU labelling. Transitional cells were analyzed by double immunofluorescence based on cytokeratin 19 and chromogranin A immunoreactivity. For purified ductal cell culture, pre-existing endocrine cells were eliminated based on their cell surface expression of N-CAM by a magnetic cell separation system (MACS).
Results: Less than 1% of the endocrine cells proliferated, mainly during the first 48 h of culture. However, a 10-fold larger proportion of the cells acquired a transitional phenotype by starting to co-express the ductal marker cytokeratin 19 (CK19). These cells represented 11 ± 2% (n=5) of all endocrine cells after one day in culture, and 6 ± 1% at 5 days of culture. After elimination of N-CAM expressing cells by MACS, we obtained 99.7% pure non-endocrine CK19-rich cell populations which could be expanded in vitro. However, the endocrine differentiation capacity of these cells was severely reduced as compared with original mixed cell cultures. Although cyst-like structures were induced, very few of the cells within the cysts started to express endocrine markers.
Conclusion: These results suggest that islet neogenesis in this culture system is at least partly representing the dedifferentiation of islet cells into a duct-cell-like phenotype, with further re-differentiation in appropriate conditions. Plasticity of differentiated human pancreatic cell types may thus be an important mechanism of human pancreas regeneration.

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Glucose-dependent expansion of pancreatic beta-cells by the protein p8 *in vitro* and *in vivo*

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Background and Aims: There is a controversy whether neogenesis from precursor cells present in ducts and islets of Langerhans or replication of beta-cells is the major source of newly formed beta-cells. In particular, molecular mediators of pancreatic beta-cell mass expansion are poorly defined. Here we report on beta-cell expansion properties of the nuclear protein p8, a member of the HMG-I/Y transcription factor family. We previously demonstrated expression of p8 in beta-cells and observed that glucose mediated INS-1 beta-cell expansion is strictly correlated to a preceeding induction of p8 protein expression.
Methods and Results: In this study INS-1 beta-cells stably overexpressing p8 protein (p8-INS-1) were generated. We find that p8 overexpression enhances INS-1 cell proliferation (3fold) without loss of mRNA expression of beta-cell phenotype related genes like PDX-1, proinsulin, GLUT2, glucokinase and amylin. Moreover, functionally mass expanded p8-INS-1 cells maintained insulin content and secretion and displayed no change in glucose stimulated insulin responses. Adenoviral overexpression of p8 in primary human islets lead to 3fold induction of cell proliferation, which cumulated in a 7fold amplification of cell numbers within one week of culture. Transplantation of these p8 transduced human islets under the kidney capsule of immunosuppressed streptozotocin diabetic mice normalized hyperglycemia more efficiently than in mice transplanted with mock transduced control islets. This was a result of p8 enhanced islet growth *in vivo* as verified by continuously rising human C-peptide serum levels and significantly elevated insulin content of p8 transduced grafts in kidney explants nine days after transplantation.
Conclusion: We conclude that p8 overexpression induces expansion of beta-cells *in vitro* and *in vivo* without loss of phenotype and function. These results establish p8 as a molecular tool for amplification of mature beta-cells for therapeutic strategies to treat diabetes mellitus.
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Hematopoietic engraftment without beta cell differentiation in the pancreas of bone marrow transplanted normal and diabetic mice

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Background and Aims: A considerable interest has developed in exploring the capacity of adult bone marrow (BM) stem cells to assume a pancreatic beta cell-like phenotype. Transplanted BM cells have been suggested to transdifferentiate into betacells at relatively high efficiency. Although this claim has been challenged, BM cells have emerged as an alternative cell source for betacell replacement therapy. In this study, adopting several alternative approaches to deliver and fate map transplanted BM cells in the pancreas of normal and diabetic mice, we sought to settle the controversy regarding this issue.
Materials and Methods: Two different GFP transgenic mouse strains were used for BM transplantation in which expression of GFP was under the control of the mouse insulin gene promoter I (MIP/GFP mice) or the general beta-actin promotor (Beta/GFP mice). The Beta/GFP mice were used to allow for specific tracking of transplanted BM cells in order to follow their migration. BM cells from either of the donor strains were transplanted into lethally irradiated wild type recipient mice (C57BL/6).
Results: The reconstitution level of the transplanted cells was above 80%. The level of GFP expression in pancreatic and liver tissue was determined after 6 weeks or 3 months in normal and diabetic mice. In normoglycemic recipient mice, a high number of GFP-expressing cells were observed in the pancreases of the mice engrafted with beta/GFP bone marrow. 99.6% of these cells were positive for the pan-hematopoietic marker CD45 and the myeoloid markers Mac-1/Gr-1. However, no cells stained positive for insulin or the beta cell transcription factors Pdx-1 and Nkx6.1. Moreover, no GFP-expressing cells were found in mice receiving BM cells from MIP/GFP mice. A total of 750 000 cells from 21 normoglycemic mice were examined. Another 21 recipient mice were treated with a single injection of alloxan (80 mg/kg) 8 weeks after transplantation to induce betacell destruction. In these mice, insulin levels decreased from 25 ± 7 μU/ml to 4 ± 2 μU/ml ($p < 0.01$) and blood glucose was elevated from 6 ± 2 mM to 15 ± 4 mM ($p < 0.05$). A subset of these mice also received 5 daily cytokine-injections 8 weeks post-transplantation in order to mobilize BM cells into peripheral blood. Similarly to the normoglycemic mice, a high level a pancreatic engraftment of transplanted beta/GFP cells was seen. Whereas 99% of these cells were positive for hematopoietic markers, no cells stained positive for beta cell markers. Furthermore, in the diabetic mice transplanted with MIP/GFP BM, not a single GFP-positive cell was observed. A total of 450 000 cells from 21 diabetic mice were investigated. Finally, a subset of the diabetic mice reconstituted with MIP/GFP BM were treated with 10 daily injections of the GLP-1 analogue exendin-4 (24 nmol/kg). However, no GFP-positive cells were found in this cohort.
Conclusion:
1.BM-derived cells efficiently engraft the pancreas in both normoglycemic and diabetic mice.
2.BM-derived cells engrafted into the pancreas are exclusively hematopoietic.
3.BM cells have little, if any, capacity to adopt a beta cell fate and are unlikely to contribute to beta cell regeneration *in vivo*.

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Hedgehog signaling: the first barrier to differentiation of pancreatic cells from ES cells?

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Background and Aims: In view of finding alternative to shortened islet cells needed for diabetes cell therapy, several studies attempted to generate pancreatic epithelial cells, namely insulin-producing cells, from embryonic stem cells during the past years. Despite tremendous efforts, protocols that allow an efficient and reproducible generation of insulin-producing cells from ES cells are yet to be set up. The strategy to be applied basically relies on features of normal pancreas development. The negative function that Hedgehog signaling fulfills in pancreatic induction and organ morphogenesis is well documented and is supported by its repression in the endoderm region that gives rise to pancreas.
Materials and Methods: We induced the E14 embryonic stem cell line to differentiate by embryoid bodies formation and assessed by immunocytochemistry and real time PCR the expression of Hedgehog pathway components in differentiating cells. Chemical and biological substances were added to cultures in order to modulate Hedgehog signaling and to determine levels of endodermal and early pancreatic genes activation.
Results: Our results point to an upregulation of ligands (Shh, Ihh), receptors (Ptc1, Smo) and intracellular effectors (Gli1, Gli2, Gli3) of hedgehog pathway upon differentiation, suggesting that Hedgehog signaling is operative in embryoid bodies and would hinder pancreatic fate acquisition. Inhibition of this pathway by Cyclopamine or a combination of Cyclopamine, Activin and Retinoid Acid allowed for further activation of early pancreas transcription factors (Pdx1, PTF1a) which were hardly detectable in control embryoid bodies. Furthermore, initial treatment of embryoid bodies by Activin A (as recently described), and to a lesser extend Activin B allowed for differentiation of endodermal cell types that can be further drove

towards a pancreatic fate. Induction of endodermal cell types by Activin A involves at least in part, a more than 100-fold downregulation of MyoR, a recognized inhibitor of endoderm differentiation in early embryo. The bottleneck of the later protocol resides in the upregulation of Hedgehog signaling (6-fold compared to maximum levels achieved in control embryoid bodies) as revealed by Shh, Ihh and Ptc1 profiles.
Conclusion: We conclude that Hedgehog signaling is operative in differentiating embryoid bodies, and is further activated by administration of Activin. As in the developing embryo, this pathway might therefore represent the first barrier to overcome if pancreatic/insulin-producing cells are to be generated from ES cells.
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Expression and function of leukemia inhibitory factor and its receptor in normal and regenerating pancreas
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Background and aims: It was recently reported that beta cell neogenesis can be induced from rat pancreatic exocrine tissue in vitro. This was based on culturing adult exocrine cells and inducing their transdifferentiation into beta cells in the presence of epidermal growth factor (EGF) and leukemia inhibitory factor (LIF). Whereas EGF is an ubiquitous growth factor expressed in many organs including the pancreas, the expression of LIF has not been studied in this organ.
Methods: We studied the expression of LIF and its receptor components, LIF-receptor and gp-130, by immunohistochemistry, western blot and RT-PCR in intact rat pancreas and in isolated tissue components thereof. We also analysed pancreas from animals that underwent duct-ligation, which was previously shown to represent an experimental model for islet regenerative growth. All experiments were performed at least 3 times independently.
Results: LIF protein was detected by immunohistochemistry in normal pancreas within the cytokeratin-positive duct cells and was absent in acinar and islet cells. LIF mRNA was also detected by RT-PCR in isolated ducts. LIF protein with an approximate molecular weight of 43 kDa was found by western blot in pancreas tissue and in ductal fluid that had been collected after occlusion of the pancreatic duct. This demonstrates that LIF is a secretory product of duct cells. The functional LIF receptor consists of the specific LIF-R and gp130. Both proteins were detected by RT-PCR in pancreas. Immunostaining revealed gp130 in the cytokeratin-positive duct cells and centroacinar cells but not in other cells. LIF-R could also be demonstrated in duct cells from human pancreas. When tissue injury and metaplasia were induced by duct-ligation, there was an upregulation of LIF and its receptor in rat pancreas. Metaplastic exocrine cells, obtained by culturing aggregated acinar cells, also started to express LIF and its receptor in vitro. Signalling via the LIF-receptor/gp130 involves the JAK/STAT pathway. Incubation of exocrine cells with LIF resulted in an increased phosphorylation of STAT3, a downstream target of the pathway. In isolated duct fragments, addition of a specific inhibitor of the JAK/STAT signalling pathway resulted in significant inhibition of duct cell proliferation as measured by BrdU-incorporation.
Conclusion: LIF is produced and secreted by cells from the exocrine ductal system of the pancreas. These cells also express functional receptors for the cytokine, indicating an autocrine or paracrine regulatory function. Our observations indicate that LIF is involved in tissue repair and in the control of duct cell proliferation. This can play a role in the regulation of beta cell regeneration.
Support: European Foundation for the Study of Diabetes (EFSD)

OP 6

Retinopathy: pathogenetic mechanisms

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Increased number of circulating endothelial progenitor cells (EPCs) in patients with type 1 diabetes and proliferative retinopathy
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Background and Aims: Proliferative diabetic retinopathy is characterized by the propagation of incompetent capillaries rapidly invading the entire retina and finally leading to retinal detachment and consequent blindness. Despite the evidence that neoangiogenesis is an established feature of the proliferative stage of diabetic retinopathy, its cellular and molecular bases are presently still unknown.
The recent identification in human adult perypheral blood of circulating endothelial progenitor cells (EPCs) that actively participate in postnatal vasculogenesis suggested the hypothesis that EPCs could be involved in the pathophysiology of the neoangiogenetic stage of diabetic retinopathy.
Materials and Methods: To clarify this issue we isolated EPCs from whole blood obtained from eleven patients with type 1 diabetes and proliferative retinopathy (the day before the first laser-therapy), twelve patients without proliferative retinopathy despite similar age, gender distribution and duration of type 1 diabetes and from eleven age- and gender-matched non diabetic controls. Peripheral blood monocyte cells (PBMCs) were isolated and seeded in petri dishes pretreated with endothelial cell attachment factor (ECAF). Cells were cultured in M199 medium supplemented with 20% bovine serum. Twenty four hours after seeding supernatant was removed, cells were spinned and re-seeded in 24-wells plates. Seven days after seeding the experiment was stopped and colonies-forming units of EPC were counted. It is commonly accepted that in these conditions EPCs are the only adherent cells forming colonies. Each colony is originated from a single EPC and therefore the number of colonies obtained is directly related to the number of circulating EPCs.
Results: As a result, we found that the number of circulating EPCs was increased in patients with proliferative retinopathy (15.4 ± 1.7 number of colonies±SE) when compared to patients without retinopathy (1.6 ± 0.7, $p=0.0001$). Non-diabetic controls showed an intermediate number of EPCs (9.5 ± 2.4). Our results also confirmed previous reports suggesting that uncomplicated diabetes is associated to a reduced number of circulating EPCs (non-diabetic controls vs diabetic patients without retinopathy: $p=0.009$). As expected, glycated hemoglobin was significantly higher in patients with proliferative retinopathy ($10.6\% \pm 0.5$ vs $8.6\% \pm 0.5$, $p=0.01$).
Conclusion: The present study demonstrates for the first time that proliferative diabetic retinopathy is characterized by an increased number of circulating EPCs. Whether this phenomenon simply parallels the intraretinal neoangiogenesis that characterizes proliferative diabetic retinopathy or is directly involved in its pathogenesis remains to be clarified by further studies.

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Effects of mechanical stress (stretch) combined with high glucose on retinal pericyte replication, apoptosis and morphology
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Background and Aims: Pericyte loss is one of the first events in diabetic retinopathy. We and others have previously showed that high glucose concentrations can induce apoptosis in cultured retinal pericytes. Both systemic and capillary hypertension are also believed to be important in the onset and progression of diabetic retinopathy (DR). The haemodynamic insult of retinal capillary hypertension can be mimicked by exposing retinal pericytes to mechanical stretch *in vitro*. Therefore, we investigated the effect of stretch combined with high glucose on both pericyte proliferation/apoptosis and morphology.
Material and methods: Bovine retinal pericytes (BRP), cultured in either normal (5.6 mmol/L) or high (28 mmol/L) glucose concentrations in six-well flexible-base plates (Flex I), were exposed or not for 48/72 hours to mechanical stress (10% elongation, 60 cycles/min). Cell replication was determined by cell count and BrdU incorporation; DNA fragmentation was measured by ELISA to assess cell apoptosis; cell morphology

and cytoskeleton distribution were evaluated by FITC-conjugated-phalloidin.
Results: Reduction of cell proliferation and increase in apoptosis were confirmed in the presence of high glucose alone. When cells were subjected to stretch, proliferation was significantly reduced in both normal and high glucose in comparison with non-stretched controls, at 48 as well as at 72 h; apoptosis was increased in both normal and high glucose at 72 h. In both cases, an important synergic effect of hyperglycaemia combined with stretch was shown. Cell morphology showed great modifications of the cytoskeleton in all experimental conditions; in particular, cells subjected to stretch showed a clear elongation and traslocation of actin fibers.
Conclusion: Our results show that stretch, alone or combined with high glucose, reduces cell proliferation, increases apoptosis and induces morphological changes in pericyte cytoskeleton. Further elucidations of the molecular mechanisms at the basis of reduced proliferation of pericytes subjected to high glucose and stretch could be useful to investigate the effects of combined hyperglycaemia and hypertension in the pathogenesis of DR.

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Gliclazide inhibits glucose- and advanced glycation end product-induced reactive oxygen species production in bovine retinal endothelial cells. Role of protein kinase C and NAD(P)H oxidase
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Background and Aims: Oxidative stress is believed to play a significant role in the development of diabetic retinopathy. Recent studies have demonstrated that metabolic factors dysregulated in diabetes stimulate reactive oxygen species (ROS) production through protein kinase C (PKC)-dependent activation of NAD(P)H oxidase in vascular cells. In the present study, we sought to determine the effect of glucose and advanced glycation end products (AGE) on ROS production in bovine retinal endothelial cells (BRECs) and the role of PKC and NAD(P)H oxidase in this effect. We further evaluated the effect of gliclazide, a second-generation sulfonylurea with antioxidant properties, on ROS generation by BRECs.
Materials and Methods: BRECs (passages 3–6) were incubated with 30 mM glucose or 100 µg/ml AGE for 24 hours. In some experiments, cells were pretreated with the PKC inhibitors, GF10923X (GF) and calphostin C, the NAD(P)H oxidase inhibitors, apocynin and diphenyleneiodonium (DPI), or the sulfonylureas, gliclazide and glyburide, prior exposure to glucose or AGE. At the end of this incubation period, cells were trypsinized and intracellular ROS production was monitored by measuring fluorescence.
Results: High glucose and AGE significantly enhanced ROS production in BRECs ($P< 0.05$). PKC or NAD(P)H oxidase inhibitors totally suppressed glucose- and AGE-induced ROS production (ROS production (% of control values): 5.6 mM glucose: 100 ± 2; 30 mM glucose: 157 ± 6, $P< 0.05$ vs 5.6 mM glucose; 30 mM glucose+GF: 104 ± 5; 30 mM glucose+calphostin C: 97 ± 4; 30 mM glucose+apocynin: 98 ± 14; 30 mM glucose+DPI: 110 ± 5; BSA: 100 ± 2; AGE: 152 ± 13, $P< 0.05$ vs BSA; AGE+GF: 101 ± 7; AGE+calphostin C: 106 ± 13; AGE+apocynin: 105 ± 6; AGE+DPI: 102 ± 12). Gliclazide decreased glucose- and AGE-induced ROS production in a dose-dependent manner with maximal effect being observed at 10 µg/ml (ROS production (% of control values): 5.6 mM glucose: 100 ± 2; 30 mM glucose: 157 ± 6, $P < 0.01$ vs 5.6 mM glucose; 30 mM glucose+2.5 µg/ml gliclazide: 158 ± 13; 30 mM glucose+5 µg/ml gliclazide: 121 ± 5; 30 mM glucose+10 µg/ml gliclazide: 116 ± 12, $P< 0.05$ vs 30 mM glucose; BSA: 100 ± 2; AGE: 143 ± 9, $P < 0.05$ vs BSA; AGE+2.5 µg/ml gliclazide: 153 ± 13; AGE+5 µg/ml gliclazide: 132 ± 10; AGE+10 µg/ml gliclazide: 91 ± 7, $P< 0.01$ vs AGE). In contrast, glyburide (2.5 µg/ml), which does not exhibit antioxidant properties, did not affect ROS production in BRECs.
Conclusion: These results demonstrate that glucose and AGE enhance ROS generation in BRECs through activation of PKC and NAD(P)H oxidase and that gliclazide inhibits this effect. These data suggest that this drug, through its antioxidant properties, may exert selective therapeutic beneficial effects in diabetic retinopathy.
Support: Servier Canada

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An antisense oligonucleotide targeting the growth hormone receptor inhibits neovascularisation in a mouse model of retinopathy
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Background and Aims: Diabetic retinopathy and age-related macular degeneration are major causes of blindness and are characterised by retinal neovascularisation. These conditions are currently treated primarily by laser ablative therapy, however this can induce retinal damage and repeated treatments are required. New therapies are in development that target some of the factors known to be involved in neovascularisation such as VEGF and IGF-I. We have previously demonstrated that a 2'-O-methoxyethyl modified antisense oligonucleotide targeted to the mouse growth hormone (GH) receptor inhibits GH binding and reduces serum IGF-I levels in normal mice. We tested this antisense inhibitor in a mouse model of retinopathy of prematurity (ROP) to determine whether a systemically delivered antisense oligonucleotide could inhibit neovascularisation in the eye.
Materials and Methods: Retinopathy was induced by housing mice across days P7 to P12 in a hyperoxic chamber (75% O_2) and was assessed at day 17 by counting blood vessel profiles in the retinas.
Results: ROP mice treated with vehicle (saline) exhibited neovascularisation levels that were 2.5-fold higher than the mean level of all sham treatment groups that did not experience hyperoxic conditions. Relative to this vehicle-treated ROP group, neovascularisation at day P17 was significantly decreased in mice treated with 5, 10, 20 or 30 mg/kg of antisense oligonucleotide, administered intraperitoneally in 5 daily doses from day P12-P16 ('late intervention'). When dosing commenced at day P7 ('early intervention'; 7 total doses, day P7, 8, 9, 11, 13, 15 and 17), significant inhibition of neovascularisation was evident in groups dosed with 5 mg/kg or 30 mg/kg antisense. The mean inhibition at the 30 mg/kg early intervention group was the maximum level of inhibition achieved across all treatment groups, and was 38% below that of the vehicle ROP group. This compared to a 26% inhibition achieved in the comparator group treated with the somatostatin inhibitor octreotide. Two control oligonucleotides with the same chemical structure were also tested in this study. There was no significant inhibition in either the early or late intervention models with control oligo at 20 mg/kg, however one of the control oligos did show a small (18%) reduction compared to vehicle ROP control when dosed at 30 mg/kg. There was nevertheless a significant reduction in neovascularisation in the corresponding 30 mg/kg antisense-treated group compared to this oligonucleotide control group, indicating a clear sequence-specific effect.
Conclusion: These results indicate that systemically delivered antisense oligonucleotides directed against the GH receptor are a potential novel therapy for neovascularisation related disorders.

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Somatostatin molecular variants in the vitreous fluid: a comparative study between diabetic patients with proliferative diabetic retinopathy and non-diabetic control subjects
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Background and Aims: There is growing evidence to indicate that somatostatin (SS) could be added to the list of natural antiangiogenic factors that exist in the vitreous fluid. In addition, a deficit of intravitreous SS-like immunoreactivity (SLI) has been found in diabetic patients with proliferative diabetic retinopathy (PDR). In the present study we have determined the main molecular variants of SS (SS-14 and SS-28) in the vitreous fluid of non-diabetic control subjects and diabetic patients with PDR in order to evaluate: a) the predominant molecular variant of SS that exists in the vitreous fluid, and b) the main SS molecular form accounting for the reduction of SLI observed in the vitreous fluid of diabetic patients with PDR. Finally, the contribution of cortistatin (CST), a neuropeptide with strong structural similarities to SS, to SLI and its levels in vitreous and plasma in both non-diabetic and diabetic patients has also been measured.
Materials and Methods: Plasma and vitreous fluid from 22 diabetic patients with PDR and 22 non-diabetic control subjects were analyzed. SS-14, SS-28 and cortistatin (CST) were measured by RIA. The antiserum employed to SS-14 determination recognized 100% of SS-14 but also cross-reacted a 31.3% for SS-28 and 30.8% for CST. By contrast, the antiserums against either SS-28 or CST were 100% specific. Consequently, SS-28 and CST were assessed by RIA, but separation by HPLC was required to measure SS-14.
Results: The predominant molecular form of SS within the vitreous fluid was SS-28 (5 fold higher than SS-14 in control subjects and 3 fold higher in PDR patients). CST significantly contributed to SLI and its intravitreous levels (pg/ml) were higher than those detected in plasma (non-diabetic controls: 146 [102–837] vs. 77 [24–32]; $p=0.01$. PDR patients: 186 [87–998] vs. 62 [24–472]; $p=0.01$). Intravitreous SS-14 was similar in both PDR sub-

jects and the control group (p=0.87). By contrast, SS-28 concentration (pg/ml) was lower in PDR patients than in non-diabetic control subjects (350 ± 32 vs. 595 ± 66; p=0.004).
Conclusion: 1) SS-28 is the main molecular variant in the vitreous fluid. 2) The intravitreous SLI deficit detected in PDR patients is mainly due to SS-28. 3) CST is abundant in the vitreous fluid and significantly contributes to SLI. These findings could open up new strategies for PDR treatment.
This study was supported by grants from Novartis Pharma S.A., the Instituto de Salud Carlos III (G03/212 and C03/08), the Ministerio de Ciencia y Tecnología (SAF2003-00550) and the Societat Catalana d'Endocrinologia i Nutrició.

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Candidate gene variant associations with diabetic microvascular disease in the EURODIAB Family Study
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Background and Aims: The purpose of this report is to describes the design and initial results from the EURODIAB Family Study (FS). The objective of the EURODIAB FS is to identify genes and pathways involved in the pathogenesis of nephropathy (DN) and retinopathy (DR) in type 1 DM. The family study involves both a family based design with affected offspring and family members and also a larger case control component that is the subject of this report. Study participants were ascertained through 28 centres across Europe some of which had participated in the EURODIAB Prospective Complications Study.
Materials and Methods: We ascertained 361 DN cases (on dialysis n=85 or persistent macroalbuminuria or elevated serum creatinine n=276), 390 DN controls (normoalbuminuria despite more than 19 years of DM and no anti-hypertensive drug use), 347 DR cases (proliferative retinopathy on retinal photo) and 368 DR controls (normal photo despite at least 10 yrs duration of DM). Our choice of candidate genes for our initial genotyping round was based on 1) previous transcriptomic work demonstrating altered expression of these genes in mesangial cells under diabetic conditions (CALD1, CTGF, GREM1, IHG1) or 2) genes encoding proteins relevant to the mitochondrial electron transport chain and oxidative metabolism of glucose, given the potential importance of this pathway in glucose induced injury (UCP2, UCP3, SOD2, SDHA, SDHB). Altogether we typed 37 promoter or exon SNPs in these genes all with previously reported rare allele frequencies >10%. Genotyping was by Taqman technology. Here we report our initial simple association analyses of these data. Ongoing analyses are focusing on haplotype analyses and detecting and adjusting for any population stratification effects.
Results: Of the 37 SNPs five had rare allele frequencies <10% such that power was minimal at these loci. None of the 37 SNPs showed any significant association with diabetic retinopathy. For diabetic nephropathy, the genotype frequency of SNP rs3807337 (A/G) in the promoter region of CALD1 was 36% 45.5% 18.5% in controls and 27% 54% 19% in cases, for AA, AG and GG respectively (p=0.028 for chi-squared test, p=0.07 for the test of linear effect of genotype, Odds ratio 1.22 by logistic regression). No other SNPs showed any evidence of association with DN. This higher frequency of the G allele in DN cases v versus controls (46% vs. 41%) at rs3807337 in CALD1 is similar to that reported previously for this SNP (48% vs 41%) and strengthens the overall evidence for its association with DN (p=0.0022 for the allele frequency comparison on the combined reported data so far).
Conclusion: The EURODIAB Family Study (FS) is an important European source of information on genetics of diabetic microvascular complications. In this initial analysis we find some supportive evidence for the role of caldesmon in DN. Caldesmon is a cytoskeletal protein that has several potential functions of relevance to DN including regulation of matrix deposition and is upregulated is mesangial cells in response to high glucose.
Support: EU 4th Framework Programme

OP 7
Oral agents, clinical aspects

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The potential of rimonabant in prediabetes: pooled 1-year results from the RIO-Lipids, RIO-Europe and RIO-North America studies
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Background and Aims: Prediabetes is associated with increased risk of type 2 diabetes (T2DM) and cardiovascular disease (CVD) and is a component of the metabolic syndrome. Rimonabant, a selective CB_1 blocker, suppresses tonic endogenous activation of the endocannabinoid system centrally promoting reductions in body weight and waist circumference, and peripherally stimulating production of adiponectin in adipose tissue and glucose uptake in skeletal muscle. Pharmacologic interventions addressing risk factors such as intra-abdominal adiposity, dysglycemia and dyslipidemia can be critical in the prevention of the progression to T2DM.
Materials and Methods: The RIO-Lipids, RIO-Europe and RIO-North America studies were multicenter, randomized, double-blind, placebo-controlled trials with similar design to assess the efficacy and safety of rimonabant (5 or 20 mg or placebo) in overweight/obese patients with the primary endpoint of weight loss and secondary endpoints including waist circumference (WC, a marker of intra-abdominal adiposity), glycemic control and lipid parameters. Pooled analysis from the three trials was conducted on 1-year data from a subgroup of patients identified with prediabetes (n=1290) as defined by impaired fasting glucose (IFG >5.5–<7.0 mmol/L [>100–<125 mg/dL]).
Results: Rimonabant 5 and 20 mg demonstrated significant weight loss, compared with placebo in the ITT prediabetes population (–3.2 kg, p=0.002; and –6.9 kg, p<0.001, respectively, vs –1.7 kg for placebo) and a similar pattern was seen for WC (–3.8 cm, p=0.001; and –6.7 cm, p<0.001, respectively, vs –2.1cm for placebo). Significant improvements were observed in the rimonabant 20 mg treatment group in HDL-C and triglyceride levels compared with placebo. Rimonabant 20 mg also reduced fasting insulin levels (–2.7 µIU/mL, p<0.001 vs placebo) and HOMA-IR (–0.8%, p=0.002 vs placebo) with a trend to reverse or retard the progression of IFG as suggested by a numerically greater percentage of patients converting to normal FPG (≤5.5 mmol/L) and a lesser proportion of patients progressing to T2DM (≥7 mmol/L) [Table]. Rimonabant 5 and 20 mg were well tolerated.
Conclusion: These data show that early intervention with rimonabant 20 mg in prediabetes improves the multiple metabolic risk factors associated with this condition. Further long-term, controlled, properly powered studies are warranted to explore the role of rimonabant in the prevention of T2DM and CVD in prediabetes.

Distribution of Fasting Glucose at 1 year: Pooled ITT population

	PLACEBO(n)	RIMONABANT 5 mg(n)	20 mg(n)
	Baseline	**Baseline**	**Baseline**
IFG	100% (290)	100% (492)	100% (508)
	1 Year	**1 Year**	**1 Year**
NFG	39.2% (105)	41.5% (186)	46.5% (218)
IFG	56.0% (150)	54.5% (244)	49.9% (234)
T2DM	4.9% (13)	4.0% (18)	3.6% (17)

IFG: Impaired Fasting Glucose; NFG: Normal Fasting Glucose; T2DM: Type 2 Diabetes
Support: Educational grant from sanofi-aventis

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Attainment of HbA_{1c} goals in type 2 diabetes patients treated with muraglitazar, a novel dual (α/γ) PPAR activator: experience from 3 large placebo-controlled trials
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Background and Aims: WHO/IDF guidelines for the treatment of diabetes establish HbA_{1c} targets of <6.5%. However, despite the availability of numerous oral antidiabetic agents, many subjects neither achieve this goal nor the less-strict <7% goal set by the American Diabetes Association.

Muraglitazar (MURA) is the first in a new class of drugs called glitazars that are dual (α/γ) PPAR activators. As an insulin-sensitizing, lipid-regulating agent, MURA simultaneously improves glycemic control and addresses the dyslipidemia characteristic of type 2 diabetes. Three separate randomized, double-blind, placebo (PLA)-controlled clinical trials evaluated MURA administered as monotherapy or in combination with either glibenclamide* (GLIB) or metformin (MET) in patients with type 2 diabetes and baseline HbA_{1c} of 7%–10%. This analysis focuses on the proportions of patients achieving accepted HbA_{1c} targets after 24 weeks of therapy.

Materials and Methods: Eligible patients were randomly assigned to once-daily treatment with MURA 2.5 mg, MURA 5 mg, or PLA for 24 weeks in addition to their baseline diabetes therapy. Subjects in Study 1 (N = 340) were drug naïve and received MURA or PLA as monotherapy; subjects in Study 2 (N = 583) had inadequate glycemic control on sulfonylurea monotherapy and received MURA or PLA in combination with once-daily GLIB 15 mg; subjects in Study 3 (N = 652) had inadequate glycemic control on MET monotherapy (1500–2550 mg/day) and received MURA or PLA while continuing a stable daily MET dose. In Study One, 109 additional subjects otherwise eligible for randomization except for HbA_{1c} levels of 10%–12% (mean 10.6%) received open-label MURA 5 mg for 24 weeks and were managed identically to the randomized subjects. The results of open-label MURA treatment complement those reported in the double-blind study.

Results: At week 24 (LOCF), 27%–58% of patients treated with MURA (alone or in combination with GLIB or MET) attained the HbA_{1c} target of <6.5% (**Table**). By comparison, 4%–14% of subjects treated with PLA (alone or in combination with GLIB or MET) had an HbA_{1c} of <6.5% at the same time point. Patients receiving either dose of MURA alone or in addition to GLIB or MET reached the <7% HbA_{1c} target consistently and substantially more often (52%–72%) than their counterparts adding PLA to their baseline regimen (13%–30%). Among patients with an HbA_{1c} of 10%–12% at screening in Study One, 28% and 39% achieved the <6.5% and <7% goals, respectively, after 24 weeks of open-label MURA 5 mg. MURA was generally well-tolerated whether administered as monotherapy or in combination with GLIB or MET.

Conclusions: The results of these trials suggest that MURA (given alone or in combination with GLIB or MET) is highly effective in lowering HbA_{1c} values to recommended goals. MURA therapy provides substantial benefits to patients with type 2 diabetes.

* US formulation = glyburide

RESPONSE AT WEEK 24 (LOCF): PERCENT REACHING HbA1c GOAL

	MURA 2.5 mg	MURA 5 mg	PLACEBO
STUDY 1: MURA/PLA	–	–	–
Baseline Mean HbA_{1c} (%)	8.0	7.9	8.0
% at HbA_{1c} Goal of			
<7%	58	72	30
<6.5%	31	58	14
STUDY 2: MURA/PLA + GLIB	–	–	–
Baseline Mean HbA_{1c} (%)	7.9	8.2	8.2
% at HbA_{1c} Goal of			
<7%	52	59	13
<6.5%	31	34	4
STUDY 3: MURA/PLA + MET	–	–	–
Baseline Mean HbA_{1c} (%)	8.0	8.0	8.0
% at HbA_{1c} Goal of			
<7%	54	64	27
<6%	27	38	10

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Efficacy of benfluorex in combination with sulfonylureas in 325 type 2 diabetic patients: a 18 week randomized double-blind study versus placebo

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Background and Aims: Lower target values for HbA1c in type 2 diabetes lead to an early use of combined oral antidiabetic agents. A common combination is sulfonylurea with metformin or thiazolidinedione. However, some patients have a limitation to the use of these agents due to a poor tolerance or a contraindication such as renal insufficiency (metformin) or cardiac failure (thiazolidinediones). Benfluorex is an agent which improves hepatic and peripheral insulin sensitivity with a mechanism of action different from that of metformin and thiazolidinedione. This is the first large scale study aimed at demonstrating the efficacy of benfluorex versus placebo as add-on therapy to sulfonylurea.

Materials and Methods: 325 type 2 diabetic patients with HbA1c: 7.0–10.0% on sulfonylurea at maximal tolerated dose and with a limitation to the use of metformin were randomized to benfluorex 450 mg/day or placebo according to a double-blind, 18 week, parallel group design. Sulfonylurea therapy was to be continued at constant dose with dietary/lifestyle advice. Main efficacy criterion was HbA1c analyzed as the change from baseline to the end of the treatment (ITT approach using and analysis of covariance with baseline ans country as covariate)

Results: The 2 treatment groups were comparable at baseline: age 63.8 ± 10.6 years, duration of diabetes 7.1 ± 6.0 years, BMI 29.4 ± 3.7 kg/m^2, HbA1c 8.33 ± 0.9%, FPG 9.8 ± 2.5 mmol/L. At the end of treatment, HbA1c was decreased on benfluorex group (n=161) from 8.34 ± 0.83% to 7.52 ± 1.04% while it was increased very slightly on placebo group (n=156) from 8.33 ± 0.87% to 8.52 ± 1.36%, with a mean adjusted difference between benfluorex and placebo of –1.01% CI 95% [–1.26; –0.76] (p <0.001). HbA1c decreased by 1% or more in 42.9% of patients on benfluorex and in 14.7% of patients on placebo (p < 0.001). Of the patients with an HbA1c of 7 to 8% at baseline, final target of HbA1c ± 7% was achieved by 47.6% versus 14.5%. The HOMA-IR index of insulin resistance decreased from 6.6 to 4.9 on benfluorex with a significant difference between groups (p <0.01). Weight was slightly decreased in both groups, significantly more on benfluorex compared to placebo (–1.3 kg on benfluorex versus –0.7 kg on placebo, p<0.01).

Overall tolerance was similar in both groups (53% of patients on benfluorex group reported at least one adverse event versus 51.3% on placebo group). Serious adverse events were more frequent in the benfluorex group, however without obvious evidence for any causality relationship. In patients with previous digestive poor tolerance on metformin, digestive side effects occurred in 15.2% of patients on benfluorex and 15.3% in placebo.

Conclusion: The addition of benfluorex is effective in achieving glycemic control in type 2 diabetic patients for whom metformin is inappropriate and who are suboptimally controlled by sulfonylurea alone. The amplitude of the effect of benfluorex on the HbA1c is within the range of that usually observed with the major oral antidiabetic drugs.

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Fluid retention and hemodilution are not associated with reduction in hematocrit and hemoglobin following pioglitazone and rosiglitazone treatment in type 2 diabetes mellitus

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Background and Aims: Thiazolidinediones (TZDs) gained widespread use for the treatment of type 2 DM. Peripheral edema, mild weight gain and anemia are often observed in TZD-treated diabetic patients. Small decreases in hemoglobin (Hb) and hematocrit (Hct) appear to be a class effect of the TZDs, although clinical symptoms related to the mild drop in Hb/Hct are very rare. The decreases in Hb/Hct are generally attributed to fluid retention, although experimental data to support such an etiology are lacking.

Materials and Methods: To examine the etiology of this mild anemia, we analyzed 62 type 2 diabetic patients (41 men/21 women, age 54 ± 1 years, BMI 29.3 ± 0.5 kg/m^2, 42 Mexican-American/20 Caucasian, duration of diabetes 4 ± 1 years, HbA_{1c} 8.4 ± 0.2%, FPG 8.5 ± 0.3 mmol/l) who have participated in randomized trials and had not previously received insulin, metformin, or a TZD. Subjects were given either placebo (n=14), rosiglitazone (ROSI, 8 mg/day) (n=15) or pioglitazone (PIO, 45 mg/day) (n=10) for 16 weeks. An additional group of 23 T2DM patients taking a stable dose of sulfonylurea (SU) for at least 3 months had either placebo (n=11) or PIO 45 mg/day (n=12) added to their SU regimen. Before and 4 months after the start of therapy, we measured total water by an IV bolus injection of ^3H_2O. Fat-free mass (FFM) was measured with ^3H_2O, bioimpedance and underwater weighing. Hb and Hct were evaluated at baseline, monthly during follow-ups and after 4 months.

Results: BMI significantly increased in all TZD-treated groups (from 28.9 ± 0.6 to 30.2 ± 0.6 kg/m^2 p<0.0001), while remained unchanged in the placebo-treated group (29.8 ± 0.8 to 29.9 ± 0.8 kg/m^2 p=NS). All of the increment (in average 3.5 kg) was accounted for by increased body fat content. Total body water did not change significantly following either TZD

(40.1 ± 1.0 to 40.5 ± 1.0 liters, P=NS) or placebo (40.7 ± 1.6 to 40.9 ± 1.6 liters, P=NS). After TZD fasting plasma glucose (–1.8 ± 0.3 mM, p<0.0001) and HbA1c (–1.5 ± 0.2%, p<0.001) declined significantly. Hb and Hct fell significantly after 4 months after TZDs (13.7 ± 0.3 to 12.8 ± 0.2, p=0.0003; 39.8 ± 0.7 to 37.1 ± 0.6%, p=0.0004, respectively). Changes in either Hct or Hb were not correlated with changes in TBW, which did not vary. White blood cell count decreased (from 6.3 ± 0.2 to 5.7 ± 0.2 x 10^3/CMM, p=0.002) in TZD-treated patients, and and platelet count fell from 210 ± 7 to 191 ± 8/CMM (p=0.06). The urinary albumin-creatinine ratio declined from 31 ± 8 to 24 ± 7 in TZD (p=0.01), indicating an improvement in renal function. No change was found in either 24-h systolic or diastolic blood pressure or in mean blood pressure measurements.

Conclusion: PIO and ROSI cause a mild anemia, which can not be explained by fluid retention. The concomitant decrease in WBC and trend toward a decrease of the platelets suggests that TZDs may exert a mild marrow suppressive effect.

Support: William K Warren Diabetes Fellow Award

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Acute effects of rosiglitazone on insulin parameters: hyperglycaemic clamp study in healthy volunteers

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Background and Aims: The insulin-sensitizing action of glitazones is mainly attributed to the activation of the peroxisome proliferator-activated receptor-gamma mesurable only after a delay of at least several days. However, studies on animal models have shown that glitazone treatment may partly exert its effects via rapidly activated alternative metabolic pathways, such as mitogen-activated protein kinase, phosphatidylinositol 3 kinase and Akt. Acute effects of glitazone treatment has never been evaluated in humans. The demonstration of such effects may have important therapeutic implications. We therefore looked for acute effects of a single dose of rosiglitazone on insulin secretion and sensitivity among healthy volunteers using the hyperglycaemic clamp technique.

Subjects and Methods: Twelve healthy male subjects, aged 27 ± 1 years, with a body mass index of 21.7 ± 0.4 $kg \cdot m^{-2}$, were included in a randomized double-blind cross-over study. Rosiglitazone (8 mg) or placebo were given orally 45 min before the hyperglycaemic clamp (blood glucose 10 $mmol \cdot L^{-1}$ during 2 h); results are expressed as means±SEM. The insulin sensitivity index was calculated as the mean glucose infusion rate from min 60 to 120 divided by the mean plasma insulin concentration during the same time period. The first phase of insulin secretion was calculated as the area under the curve of plasma insulin from min 0 to 10. The second phase of insulin secretion was calculated similarly from min 10 to 120. Comparisons of means were performed by repeated measures ANOVA with assessment of carry-over effect, after logarithmic transformation.

Results: The mean plasma rosiglitazone concentration peaked exactly at the start of the hyperglycaemic clamp and reached 415 ± 96 mg/L. The mean glucose concentration during the clamp was 10.2 ± 0.2 $mmol \cdot L^{-1}$. The insulin sensitivity index was significantly increased by rosiglitazone: 12.0 ± 1.5 vs. 8.5 ± 1.1 $\mu mol \cdot m^{-2} \cdot min^{-1} \cdot pmol^{-1} \cdot L$ in the control period (P <0.01). Accordingly, the second phase of insulin secretion was significantly decreased in the presence of rosiglitazone: 13066 ± 1531 vs. 16316 ± 2813 $pmol \cdot L^{-1}$ x 110 min in the control period (P <0.05). In contrast, the first phase of insulin secretion was not modified by rosiglitazone. No carry-over effect was observed for any of the variables tested.

Conclusion: The present results show for the first time that a single dose of rosiglitazone induces an immediate increase in insulin sensitivity in non diabetic young male volunteers. The decrease in the second phase of insulin secretion is related to this improved insulin sensitivity. The precise mechanisms mediating this action remain to be determined.

Support: Montpellier University Hospital, INSERM and Glaxo Smith Kline

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The risk of cancer in thiazolidinedione users compared to other antidiabetic agents

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Background and Aims: There are preliminary data on the potentially beneficial role of Peroxisome proliferator-activated receptor (PPAR) gamma agonists including Thiazolidinediones (TZDs) in reducing the risk of future cancer and/ or as anti-cancer therapy. On the other hand, an increased incidence of certain cancers has been reported in 2 year rodent carcinogenicity studies with some PPAR agonists. The primary objective of this study is to determine if the likelihood of developing breast, colon, and prostate cancers is different in patients exposed to TZDs compared to other antidiabetic agents.

Materials and Methods: A case-control study was conducted to determine whether TZDs alter the risk of breast, colon and prostate cancers compared to traditional antidiabetic agents. Incident cases of cancer among type 2 diabetic patients were identified from the Integrated Healthcare Information Services (IHCIS) managed care database from 1997 and 2002. Incident cases of each of the cancers of interest were defined as subjects with an ICD-9 code for the specific cancer (Breast cancer ICD-9= 174 malignant neoplasm of female breast; Prostate cancer ICD-9 = 185 malignant neoplasm of prostate and ICD-9 = 187.8 seminal vesicles; Colon Cancer ICD-9 = 153 malignant neoplasm of colon), occurring at least three months after the subject study period begin date. Patients with the prior cancer(s) of interest were excluded. One control was matched to each cancer case on age, gender and calendar year of cancer diagnosis (index year).

Subjects exposed to TZDs (Rosiglitazone, pioglitazone) monotherapy or in combination with other oral agents &/or insulin in the three months prior to the index date were compared to subjects exposed to all other antidiabetic agents. The odds of the specific cancers of interest were modeled using conditional logistic regression, adjusting for age, gender and index-year of cancer diagnosis.

Results: We identified 672 incident prostate cancer cases among 130,000 diabetic male patients, 450 colon cancer cases among 230,780 diabetic patients, and 701 breast cancer cases among 100,780 female diabetic patients. Table 1 gives the odds ratios comparing the likelihood of prostate, colon and breast cancer in TZD users compared to other antidiabetic agents. Compared to other antidiabetic agents, there is no significant increase or decrease in the likelihood of prostate, colon and breast cancer in TZD exposed patients compared to other antidiabetic agents.

Conclusion: Our findings suggest that the effect of TZDs on the likelihood of development of the cancers studied (colon, prostate and breast) appears to be neutral and do not support a beneficial or deleterious effect of TZD on the cancers studied, as suggested by biological experimental models.

Table 1: Odds Ratio, 95% CI and p-value for the cancers of interest in TZD users compared to other antidiabetic agents:

	Odds Ratio (95% CI)	p-value
Prostate Cancer	1.01 (0.75–1.37)	0.945
Colon Cancer	0.90 (0.62–1.32)	0.601
Breast Cancer	0.92 (0.57–1.50)	0.736

OP 8
Predictors of type 2 diabetes

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Birth weight and risk of type 2 diabetes: a meta-analysis
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Background and Aims: Low birth weight is suspected to be a risk factor for the development of type 2 diabetes in later life. However, there are also reports which found high birth weight to be associated with type 2 diabetes. We performed a comprehensive meta-analysis of all published studies on birth weight and later risk of type 2 diabetes.
Materials and Methods: A systematic literature review was conducted including the databases MEDLINE (1966–June 2004) and EMBASE (1989–June 2004), review papers and reference lists of published papers. Studies which reported odds ratio (OR) and 95% confidence interval (95% CI, or data to calculate them) of type 2 diabetes associated with birth weight were included. Study estimates were pooled using fixed effects and random effects model.
Results: 13 studies met inclusion criteria. Low birth weight (<2500 g) was associated with an increased risk of type 2 diabetes in later life (OR: 1.31; 95% CI: 1.05–1.65). To a similar degree, high birth weight (>4000 g) was found to be associated, although non-significantly, with increased risk of type 2 diabetes (OR: 1.26; 95% CI: 0.99–1.61). Influence analysis indicated that these pooled estimates are highly instabil, due to marked discrepancies between the estimates of larger compared to smaller studies. Most noteworthy, however, only two of the 13 studies considered gestational age. While one of these two studies reported low birth weight to be associated with increased risk of type 2 diabetes (unadjusted relative risk: 1.83, 95% CI: 1.55–2.16), the other reported high birth weight to be a risk factor for type 2 diabetes (unadjusted OR: 2.53, 95% CI: 1.66–3.87). Both studies did not find a significant influence of gestational age (full-term subjects only: relative risk: 1.75, 95% CI: 1.51–2.03; full-term subjects only: OR: 2.42, 95% CI: 1.42–4.13).
Conclusion: The data of our meta-analysis indicate that the relation between low birth weight and risk of type 2 diabetes is strongly overemphasized currently, while gestational age has largely been ignored so far. Moreover, the so-far published data show that high birth weight is associated with increased risk of type 2 diabetes in later life at least to the same extent as low birth weight. More research is needed to further characterize the size of the effect, possible confounders and etiopathogenetic mechanisms responsible for these associations.
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Value of isolated impaired fasting glucose for the prediction of the incidence of type 2 diabetes
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Background and Aims: Considering the cumbersome nature of the oral glucose tolerance test (OGTT), fasting glucose measurements are often used as a first indicator of the risk of diabetes in clinical practice. However, the significance of an isolated impaired fasting glucose (IFG) value for the risk of future diabetes is still under debate. We aimed to compare the value of an isolated IFG, and IFG in conjunction with other diabetes risk factors for the prediction of new cases of type 2 diabetes over 10 years of follow up in 5914 participants of the Whitehall II study.
Methods: Participants of the Whitehall II study (72% men; mean age 49.3 years, SD 6.0) underwent three full cardiovascular risk assessments, including OGTT, at 5-year intervals. For the current analyses, participants with known diabetes at baseline were excluded and OGTTs at 5 and 10 years of follow-up were used to identify new cases of type 2 diabetes.
Results: At baseline, the prevalence of IFG on a fasting glucose test was 3.2% according to the WHO classification and 14.7% according to the ADA classification. Oral glucose tolerance tests at follow up identified 272 new cases of diabetes (4.6%), of which 18.0% and 37.1% had baseline IFG according to WHO and ADA respectively. In isolation, IFG at baseline predicted incident new diabetes with an odds ratio (OR) of 8.57, 95%CI 6.03–12.18 (WHO classification) and 3.76 (2.91–4.86) (ADA classification). Adjustment for BMI, hypertension, family history of DM, total serum cholesterol, HDL cholesterol and physical inactivity, yielded an OR of 7.04 (4.86–10.19) and 3.06 (2.33–4.03) for the WHO and ADA classifications respectively. The predictive performance of an isolated IFG assessment, expressed as the area under the ROC curve was 0.58 (0.54–0.62) according to the WHO classification and 0.62 (0.58–0.66) according to the ADA. A model including all variables mentioned above except IFG, predicted the incidence of diabetes significantly better than the isolated IFG assessment (area under ROC 0.69 (0.66–0.72). Adding IFG to this multivariable model further increased the predictive value (area under ROC 0.73 (0.70–0.76) for either classification).
Conclusion: The apparent discrepancy between the marked risk associated with IFG and its relatively modest contribution to the over-all predictive performance of a multivariate model is explained by the small proportion of the population who have IFG. As the elevated risk conferred by IFG applies only to this minority, future diabetes is best predicted by other risk factors in a majority of the population. Our results indicate that an isolated IFG assessment is a poor predictor of future diabetes compared to either a model based on multiple diabetes risk factors or a model where risk factors and IFG are considered jointly. A fasting glucose value should therefore be seen as a useful component of a set of risk factors rather than as a risk indicator in isolation. When such a multivariable approach is taken, both WHO and ADA classifications yield an equal level of prediction.
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The metabolic syndrome, risk scores and glucose values in predicting incidence of type 2 diabetes over six years – The Hoorn Study
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Background and Aims: Numerous investigators have proposed risk scores to predict diabetes, and recent studies have shown a strong association of metabolic syndrome and diabetes risk. We used population-based data to assess risk scores, glucose values and the metabolic syndrome in predicting 6-year incidence of type 2 diabetes in Dutch men and women.
Materials and Methods: In the Hoorn region of the Netherlands, 2484 subjects, aged 50–75 years, were recruited to participate in a cohort study with follow-up by oral glucose tolerance test six years later. We used logistic regression to estimate risk of incident diabetes in 601 and 724 women with data at both examinations. Various risk algorithms, metabolic syndrome criteria (NCEP), and the number of metabolic syndrome components were used as independent variables.
Results: Fasting and postprandial glucose values were strongly predictive of 6-year incident diabetes (129 cases), with estimated area under ROC curves of 0.79 and 0.65, respectively. The Cambridge risk score also showed a strong relationship to incident diabetes in both genders. BMI and waist circumference were significant only in women. Men and women with NCEP criteria were 2 and 6 times more likely to develop diabetes, respectively, compared to those without metabolic syndrome. A modification requiring waist circumference and at least two other components and using a lower cutpoint for fasting glucose, showed somewhat attenuated odds ratios which were weaker than IFG and IGT status. The number of metabolic syndrome components was predictive, with a 1.94 and 2.44-fold risk in men and women respectively, for each increase in number of components present, compared to those with no components. In multivariate models including fasting glucose, 2-hour glucose statistically contributed but to a lesser degree in predicting diabetes, while Hba1c added little in men. In addition, the metabolic syndrome criteria and number of components present significantly predicted diabetes in women with and without impaired fasting glucose, but the number of metabolic syndrome components present was significant in men only in those with normal fasting glucose.

Odds Ratios@ (95% CI) for Diabetes, Adjusted for Age and Duration of Follow-up

Algorithms/Scores	MEN (n=601)	WOMEN (n=724)
Fasting glucose (FG)	10.19 (5.47–18.97)	6.84 (3.95–11.85)
2 hour glucose	1.63 (1.39–1.91)	1.99 (1.68–2.35)
HbA1c	2.08 (1.16–3.71)	6.13 (3.19–11.79)
BMI	1.02 (0.92–1.13)	1.14 (1.06–1.22)
Waist circumference	1.03 (0.99–1.06)	1.08 (1.05–1.10)
ADA risk score+	1.00 (0.92–1.08)	1.09 (1.00–1.18)
Cambridge score+ (per 0.10 inc. in probability)	1.18 (1.05–1.34)	1.31 (1.16–1.47)
NCEP ATP III metabolic syndrome criteria	2.93 (1.67–5.15)	6.02 (3.48–10.42)
Number of metabolic syndrome components	1.94 (1.54, 2.44)	2.44 (1.93, 3.08)
NCEP, waist required, lower cut on FG	2.35 (1.17–4.75)	5.75 (3.32–9.96)

@per unit increase in algorithm +Other risk scores showed comparable results

Conclusion: Fasting glucose levels and the Cambridge risk score were strong predictors of diabetes in Dutch men and women. In the absence of fasting glucose levels, certain risk scores may be useful in predicting risk of future diabetes. The metabolic syndrome criteria and number of components present appears to provide useful predictive information for diabetes in women with and without impaired fasting glucose, while the number of components present appears to be useful in men without impaired fasting glucose.

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One year follow-up in a high risk population in general practice on the progression from IGT and IGF to diabetes, ADDITION DK

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Background and Aims: Lifestyle intervention in persons with IGT reduces the progression to diabetes. The progression rate from IGT to diabetes in population based studies is 4–9% and higher in the control group in recent intervention trials (7–13%). Little is known about why some individuals progress from IGT to diabetes and even less is known with respect to progression from IFG to diabetes. The aim of this study is to estimate the progression rates to diabetes from IGT and IFG in a high risk population identified by a high risk screening strategy for diabetes in general practice.
Materials and Methods: In Denmark, 125,000 persons in the age of 40–69 years have so far been enrolled in the ADDITION study screening for T2DM in general practice based on a high risk, stepwise strategy. The screening process also identifies individuals with elevated fasting blood glucose (IFG) and/or 2 hour blood glucose after a standard oral glucose tolerance test (IGT). No specific glucose lowering treatment is initiated towards individuals in this category but the general practioners are recommended to advice the patients for yearly examinations of glucose tolerance status hereafter. After one year the patients are invited to make an appointment with their general practioner for a follow-up on the glucose tolerance status. 1187 persons were identified with IGT or IFG among the high risk persons screened from April 2001 up to August 2003. Follow-up after one year was registered for 822 persons (69.3%).
Results: Using WHO1999 criteria, the baseline classification of the 822 persons was n=511 with IGT and n=311 with IFG.

	n	Women (%)	Mean age (years)	Progression rate (DM cases/100 person-years)
IFG	311	39.9	59.8	17.7
IGT	511	55.2	61.3	19.0

Progression rate for IGT was 19.0 / 100 person-years (95%CI (15.7–22.7)) and for IFG 17.7 / 100 person-years (13.8–22.2) (p>0.05). No difference in progression rates between women and men was observed. BMI, known hypertension, FBG and 2 hBG were associated with progression from IGT, and for IFG BMI and systolic blood pressure were associated with progression to diabetes.
Conclusion: High risk screening strategies for diabetes also identifies individuals with IGT and IFG. Persons with IGT or IFG identified by the present high risk strategy demonstrated progression rates to diabetes even higher than reported for the control groups in the recent intervention studies. The progression rates for IGT and for IFG were of the same magnitude. Thus this study indicates that individuals with IGT or IFG identified as part of high risk screening strategies are at particular high risk of progression to diabetes, and thus that lifestyle based intervention should be encouraged in these groups.

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Characteristics of insulin secretion (OGTT) in the Japanese subjects with impaired fasting glucose and/or impaired glucose tolerance; The Ichihara diabetes study

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Background and Aims:The aim of this study was to clarify the characteristics of insulin secretion after glucose load in the Japanese subjects with impaired fasting glucose and/or impaired glucose tolerance.
Materials and Methods:We did a screening test of 22228 general healthy subjects aged over 40 years old from Ichihara-city. In the 1st. screening test, the subjects were screened for the criteria of glucosuria, FPG levels (110 mg/ml) and/or HbA1c levels (5.6%). In the second test (OGTT), GTT data from 468 cases applying for a close examination among the positive subjects in the 1st. test were analyzed in detail. Based on the results of 75 g OGTT, the subjects were classified into the following five groups:normal glucose tolerance (NGT), isolated impaired fasting glucose(IFG: FPG between 110 mg/dl and 125 mg/dl, 2 hPG less than 140 mg/dl), isolated IGT (FPG less than 110 mg/dl and 2 hPG between 140 mg/dl and 199 mg/dl), isolated IGT with fasting hyperglycemia (IFG/IGT: FPG between 110 mg/dl and 125 mg/dl, 2 hPG between 140 mg/dl and 199 mg/dl) and DM groups. The DM group is further divided into 3 groups, isolated FPG-DM group (FPG not less than 126 mg/dl and 2 hPG less than 200 mg/d), isolated 2 hPG-DM group (FPG less than 126 mg/dl and 2 hPG not less than 200 mg/dl) and complete DM group (FPG not less than 126 mg/dl and 2 hPG not less than 200 mg/dl).
Results:1) In the GTT study,DM was found in 23.5%, NGT:41.9%, isolated IFG: 6.2%, and IGT (Isolated IGT+IFG/IGT) was found in 28.4% (Details: Isolated IGT: 20.3%, IFG/IGT: 8.1%).The incidence of the subjects with isolated IFG was much lower than that of the subjects with IGT. 2) Insulinogenic index (30 min): (Ins 30 min–Ins 0 min)/Glu30 min–Glu 0 min) in the DM group was significanntly lower than that in the NGT group. ($p < 0.05$ DM: 0.22, NGT: 0.53).The index of the IGT subjects was lower than that of the NGT group (IGT: 0.46). In the IGT subjects, Insulinogenic index (30 min)in the IFG/IGT group was significanntly lower than that of the isolated IFG group ($p<0.05$: IFG/IGT 0.30 vs Isolated IFG 0.64) and lower than that of the isolated IGT group (Isolated IGT 0.57), but the subjects with low levels of Insulinogenic index (less than 0.4) were found in 57.1% in the isolated IFG group. In the DM group, the index of the isolated 2 hPG-DM group was significantly lower than that of the isolated FPG-DM group ($p<0.01$: 0.15 vs 0.51) and significantly lower than that of the IFG/IGT and the isolated IFG groups ($p< 0.05$: 0.15 vs IFG/IGT 0.30 and $p<0.001$: 0.15 vs Isolated IFG 0.64) 3). Basal insulin levels were as follows: Isolated IFG: 10.3, IFG/IGT: 10.1, Isolated IGT: 6.5, NGT: 5.9, Isolated 2 hPG-DM: 8.6uU/ml. Basal insulin level in the IFG/IGT group was significantly higher than that in the NGT and the isolated IGT groups ($p < 0.001$ and $p < 0.01$). Basal insulin level in the 2 hPG-DM was significantly higher than that in the NGT and isolated IGT groups($p < 0.001$ and and $p < 0.05$).
Conclusion:In the Japanese subjects, the incidence of the subjects with isolated IFG is much lower than that of the subjects with IGT. And the pattern of insulin secretion in the Japanese IGT subjects including IFG/IGT is characterized by marked low responses in the early-phase of insulin secretion compared to those in Caucasian IGT subjects. The development from NGT via IGT to 2 hPG-DM and type 2 DM is considered mostly due to the decrease of early-phase insulin secretion in the Japanese subjects.

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Single nucleotide polymorphisms in the promoter of *HNF4A* predict the conversion from impaired glucose tolerance to type 2 diabetes: the STOP-NIDDM trial

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Background and Aims: Hepatocyte nuclear factor 4 α, an orphan nuclear receptor encoded by *HNF4A* gene, plays a crucial role in regulating hepatic gluconeogenesis as well as the development, differentiation and function of the pancreatic β-cells. Mutations in the coding region of *HNF4A* have been shown to cause the maturity onset diabetes of the young (MODY 1). Recently, single nucleotide polymorphisms (SNPs) of *HNF4A* have been shown to be associated with Type 2 diabetes in Finns and Ashkenazi Jews. These results were later on confirmed in Caucasians from UK, and in Amish and Danish subjects. Aiming to replicate and extend these findings, we selected five SNPs of P1 (rs1885088 G/A and rs3818247) and P2 (rs4810424 G/C, rs1884614 C/T and rs2144908 G/A) promoter regions of *HNF4A*, and investigated whether they predict the development of type 2 diabetes in subjects with impaired glucose tolerance (IGT) participating in the Study To Prevent Non Insulin Depending Diabetes Mellitus (STOP-NIDDM).
Materials and Methods: The STOP-NIDDM trial, including 1429 subjects with IGT, was a longitudinal, double blind, placebo-controlled randomised trial aiming to evaluate the effect of acarbose, compared to placebo, in the prevention of type 2 diabetes. The TaqMan Allelic Discrimination Assays were used to genotype SNPs in *HNF4A* in 769 DNA samples available.
Results: SNPs in the P2 promoter region of *HNF4A* were found to be in a strong linkage disequilibrium. Female carriers of the rare alleles of either SNP:rs4810424 (C allele) (OR=1.65, 95% CI 1.07–2.55; p=0.023), SNP:rs1884614 (T allele) (OR=1.64, 95% CI 1.06–2.53; p=0.025) or SNP:rs2144908 (A allele) (OR=1.57, 95% CI 1.02–2.43; p=0.040) had an elevated risk for the conversion to diabetes compared to subjects with the common genotypes. The presence of the rare T allele of the SNP:rs3818247 was associated with a 1.68-fold higher conversion rate to diabetes in the acarbose group (95% CI 1.07–2.64, p=0.024). Additionally, in the entire population the AA genotype of SNP:rs2144908 increased the risk for diabetes by 1.73-fold (95% CI 1.02–2.95; p=0.045) when compared to subjects with the GG genotype. Adjustment for age, treatment group, gender, smoking, weight at baseline and weight change did not change the results.
Conclusion: Our results further suggest that single nucleotide polymorphisms in *HNF4A* modify the risk for type 2 diabetes.
The STOP-NIDDM trial and DNA analyses were funded by an unrestricted grant from Bayer AG

OP 9
Diabetes in pregnancy

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Umbilical cord blood leptin concentrations in average for gestational age and small for gestational age babies of nondiabetic and diabetic mothers

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Background and Aims: Cord blood leptin is now thought to be a major determinant of intrauterine fetal growth. The interaction of leptin and insulin in case of babies of diabetic mothers still needs to be clarified in various groups due to the dependence of both the hormones on genetic and environmental factors. As a part of an attempt to clarify these issues we have studied the interaction of cord blood leptin with intrauterine growth of babies from diabetic and nondiabetic mothers.
Materials and Methods: Umbilical cord blood was obtained (from placental side of umbilical cord after double clamping of the cord) from 32 small for gestational age babies from type 2 diabetic mothers (DSGA- babies), and 22 babies from nondiabetic mothers (NDSGA - babies) as well as from 84 average for gestational age babies of diabetic (DAGA- babies) and 28 babies of nondiabetic (NDAGA - babies) mothers of term pregnancy (age of all mothers: 25–35 years). Weight of the baby was measured by weighing balance. Serum leptin level was measured by chemiluminescence-based ELISA (DPC, USA).
Results: The DSGA group showed lower levels of leptin [cord blood leptin, μIU/ml, median (range), 6.85 (2.0–20.56)] as compared to DAGA group [28.20 (23.00–81.20), p<0.001]. The NDSGA group also showed lower levels of leptin [5.94 (3.2–11.0)] as compared to NDAGA group [20.93 (11.0–47.6), p<0.001]. The DAGA group showed significantly higher cord leptin level [DAGA, 28.20 (23.00–81.20)] compared to the NDAGA group [20.93 (11.0–47.6), p=0.018]. No significant difference of leptin was found between DSGA group [6.85 (2.0–20.56)] and NDSGA group [5.94 (3.20–11.0)].
Conclusion: Differences in leptin concentrations between babies of nondiabetic and diabetic mothers exist for average weight for gestational age group but not for small for gestational age babies. The mechanism underlying this difference, particularly the role of insulin, needs to be explored.

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Plasma ghrelin relates to the degree of obesity and insulin resistance in women after gestational diabetes

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Background and Aims: Circulating hormones like ghrelin, secreted by gut cells, and leptin, stemming from the white adipose tissue, form a close network with the central nervous system, providing a feeback loop between energy intake and regulation of body weight. Decreased plasma ghrelin (PG) and increased plasma leptin associated with leptin resistance characterise obese or insulin-resistant patients. We aimed to investigate early changes in PG and its interrelationship with insulin sensitivity, adipocytocines and anthropometric parameters in women with prior gestational diabetes (pGD), where subtle disturbancies in glucose metabolism persist after delivery.
Materials and Methods: 69 pGD and 15 glucose tolerant women during gestation (CON, BMI- and age matched), underwent an intravenous glucose tolerance test (FSIGT) for evaluation of the insulin sensitivity index (S_I) and an oral glucose tolerance test (oGTT 75g/180 min) 18–36 months after delivery; the disposition index (DI, oGTT: $OGIS \times \Delta AUCI_{0-30}$) did not differ between groups, whereas plasma glucose was increased in pGD (fasting: 96.5 ± 2.1 vs. 82.7 ± 1.1 mg/dl, p <0.0001; 2 h: 134.0 ± 6.5 vs. 98.5 ± 4.4 mg/dl, p<0.006, vs. CON); PG was taken in timed intervals (oGTT), analysis with a commercial RIA (Peninsula Labs, San Carlos, CA); plasma leptin concentrations (PL) and PAI-1 were collected in the fasting state; all women were also studied according to their tertiles of the body fat mass (FM: 1rst Tertile (T1) <19.8 kg; 3rd Tertile (T3) ≥28.5 kg; bioelectrical impedance analysis).
Results: PG did not differ between pGD and CON (208.1 ± 31.2 vs. 223.9 ± 42 fmol/ml). In CON PG only related inversely to the degree of obesity (FM: r= –0.61, p<0.04) and PL (r= –0.61, p<0.03), whereas in the total

group PG was in addition to FM (r=–0.36, p<0.005), and PL (r=–0.26, p<0.05) also associated with glycemic parameters (DI: r=0.40, p<0.004; HbA1c: r=–0.27, p<0.003) and lipids (TG: r=–0.22, p<0.04; HDL: r=0.25, p <0.02). In pGD PG also correlated with insulin sensitivity (S_I: r=0.31, p <0.03). After correction for FM, PG still correlated with metabolic control (DI: r=–0.81, p <0.0003 in pGD; HbA1c: r= –0.98, p <0.03; fasting glucose: r= –0.98, p <0.006 and PAI-1: r= –0.98, p <0.05 in all women). Women with highest amount of FM (T3) had lowest PG (p <0.04, vs. T2 and T1). Accordingly, insulin-resistant pGD had lower PG ($PG_{fasting}$: 165.0 ± 33.0 vs. 234.0 ± 21.8 fmol/ml, p<0.01) than those with normal insulin sensitivity.
Conclusion: Plasma concentrations of the orexigenic hormone ghrelin are highly correlated to parameters of glucose metabolism and cardiovascular risk factors in particular in pGD women as well as to the body fat content and the anorexigenic hormone leptin in all women 18–36 months post partum. Particularly in women at increased risk for type 2 diabetes ghrelin might have integrative functions linking obesity to insulin resistance and glucose and lipid metabolism.

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Plasma adiponectin levels are reduced in women with prior gestational diabetes

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Backgrounds and Aim: Plasma adiponectin levels are reduced in insulin resistant state such as type 2 diabetes mellitus, dyslipidemia and atherosclerosis. Since women with prior gestational diabetes (pGDM) are insulin resistant, we have determined adiponectin levels and their association with cardiovascular risk factors.
Matherials and Methods: A total of 116 pGDM women and 30 women with normal glucose tolerance during and after pregnancy (CON) were evaluated 24 months after delivery. Following OGTT (75-g), pGDM women were classified as normotolerant (NGT, n=88) or impaired glucose regulation (IGR: altered fasting and/or postload glucose tolerance; n=28). In each women we determined antropometric parameters, blood pressure, plasma glucose, insulin, lipid profile, adiponectin, high-sensitive C-reactive-protein (hs-CRP), fibrinogen. Insulin sensitivity index (ISI-Matsuda) was calculated.
Results: The three groups were comparable for BMI, age, waist circumference and mean blood pressure. Insulin sensitivity progressively decreased from CON to NGT and to IGR, (8.6 ± 4.1 vs 5.8 ± 3.1 vs 4.0 ± 2.1; ANOVA p<0.001). HDL-cholesterol levels were similar in NGT (53.81 ± 13 mg/dl) and IGR (53.50 ± 14 mg/dl) though both were lower than those found in CON (60.8 ± 12 mg/dl, p<0.05). Adiponectin levels were comparable in NGT (6.6 ± 2.7 µg/dl) and IGR (6.2 ± 2.7 µg/dl) and were lower than CON (8.1 ± 3.3 µg/dl, p<0.01). hs-CRP was higher in IGR (4.1 ± 3.4 mg/dl) than NGT and CON (1.6 ± 2.5 and 1.7 ± 2 mg/dl, p<0.05). To rule out the confounding effect of obesity, analysis was performed in women with BMI<25 kg/m^2, leaving 47 NGT pGDM (BMI: 21.2 ± 2.3), 19 IGR (BMI: 21.9 ± 1.4) and 23 CON (BMI: 21.1 ± 1.9 kg/m^2). ISI was similar in IGR and NGT (4.7 ± 2.5 and 6.9 ± 3.3, respectively), but lower than CON (9.3 ± 4.1, p<0.05). Similarly adiponectin was comparable between IGR and NGT (6.7 ± 2.3 and 7 ± 2.8 µg/dl) but lower than CON (8.56 ± 3.3 µg/dl, p<0.05). No differences were found as the other parameters were concerned.
In the whole population, after multivariate analysis, adiponectin was positively correlated with HDL cholesterol (p<0.001, r=0.5) and inversely with tryglicerides (p<0.001, r=0.6). ISI was correlated with systolic blood pressure (p< 0.01, r=0.3), HDL-cholesterol (p<0.001, r=0.4) and inversely with adiponectin (p<0.001, r=0.5).
Conclusions: Low plasma adiponectin levels characterize women with prior GDM independently of adiposity and glucose tolerance. Adiponectin can be taken as a marker of insulin resistance and increased cardiovascular risk also in pGDM women.

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Fasting blood glucose in the postpartum period is the best predictor for glucose tolerance abnormalities in women with prior gestational diabetes

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Background and Aims: Impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) are frequently not coincident and, probably, respond to different fisio-pathologic mechanisms. If IFG precedes or is a less-serious condition than IFG is also not known. The study aim was to characterize in the postpartum period glucose tolerance abnormalities in relation to insulin resistance and other components of the metabolic syndrome.
Materials and Methods: 367 post-partum women, 280 with prior gestational diabetes (GD group, age 33.8 ± 4.3 years-old, BMI 27.1 ± 5.5 kg/m^2, waist width 83.9 ± 11.8 cm) and 87 without GD (control group, 32.4 ± 4.1 years-old, BMI 25.5 ± 4.1 kg/m^2, waist width 79.7 ± 9.7 cm) were included in the study. Women were classified after a 75g-glucose OGTT as follows: 1) normal (FBG <110 mg/dl and 2 h-OGTT <140 mg/dl); 2) IFG (FBG ≥110 but <126 mg/dl and 2 h-OGTT <140 mg/dl); 3) IGT (FBG <110 mg/dl and 2 h-OGTT ≥140 mg/dl but <200 mg/dl); 4) IFG+IGT (FBG ≥110 but <126 mg/dl and 2 h-OGTT ≥140 mg/dl but <200 mg/dl) and 5) diabetes mellitus (DM, FBG ≥126 mg/dl or 2 h-OGTT ≥200 mg/dl). Besides anthropometric variables, HOMA-IR, OGIS, HOMA-estimated β-cell function and 30-min insulin increase after a glucose challenge were compared. Statistical comparisons were performed using chi square, one-way ANOVA and Tuckey's tests, with a significance level of < 0.05.
Results: In the postpartum period, the prevalence of glucose abnormalities was higher in DG group (27.9% with IFG, IGT, IFG+IGT or DM) as compared to the control group (10.3% with IFG or IFG+IGT), chi-square=11.3, p=0.001, OR=3.35, IC 95% 1.60–7.00. Insulin resistance by HOMA-IR was similar in women with DM (3.8 ± 2.9), IFG (3.9 ± 2.5) or IFG+IGT (4.3 ± 2.0) but lower in control (1.9 ± 1.5) and IGT women (1.7 ± 0.8), p<0.001. Using the OGIS index, lower values were determined for DM (321.3 ± 42,4) and IFG women (355.9 ± 47.5), intermediate for IFG+IGT (349.4 ± 47.5) and IGT women (395.0 ± 42.2) and higher for control women (435.1 ± 54.6, p<0.001 compared to other groups). Apart from the control group, only women with IGT were different from DM using the OGIS index, p<0.001. Anthropometric variables (BMI and waist width) followed the same pattern as the OGIS comparisons. No differences between the groups were observed using insulin secretion tests, either HOMA-estimated β-cell function or 30-min insulin increase after glucose challenge.
Conclusion: In the postpartum period, IFG, alone or in combination, identified more accurately those women which are more insulin resistant and had typical anthropometric changes of the metabolic syndrome. In view of our results, the need to perform a 75-g glucose OGTT after delivery to characterize glucose tolerance abnormalities in women with prior GD seems questionable.

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Preconceptional HbA_{1C} and risk of severe adverse pregnancy outcome in 966 women with type 1 diabetes – a graphical approach

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Background and Aims: Glycaemic control is related to severe adverse pregnancy outcomes, and it is not known, whether there is a threshold value for HbA1c above which the risk increases substantially. The aim was to illustrate the relationship between preconceptional HbA1c and risk of congenital malformations (CM) and/or perinatal mortality (PM) in type 1 diabetic pregnancies using a graphical method.
Materials and Methods: Population-based nation-wide study of 1215 consecutive type 1 diabetic pregnancies. Recurrent and multiple pregnancies were excluded leaving 966 pregnancies for analysis. Frequencies of CM and PM were related to preconceptional HbA1c (Number of SD's above normal mean = Z-score) by linear and quadratic logistic models (cubic spline).
Results: The risk of severe adverse outcomes (PM and CM combined) tended to increase at even slightly raised HbA1c values and was markedly raised when HbA1c values exceeded mean + 10 SD. However, the number of women with extremely high HbA1c was small giving less accurate esti-

mates in these groups. Pre-gestational HbA1c was more predictive for PM than HbA1c in later pregnancy. Frequencies for CM and PM are given below. Spline-curves will be presented.

No. of women[§]	HbA1c (%)[*]	Z-score (SD's above mean)	CM% (95% CI)	PM% (95% CI)
55	≥ 10.4	≥ 10	10.9 (4.1–22.3)	5.5 (1.1–15.1)
128	8.9–10.3	7.0–9.9	3.9 (1.3–8.9)	6.3 (2.7–11.9)
182	7.9–8.8	5.0–6.9	5.0 (2.3–9.2)	3.3 (1.2–7.0)
284	6.9–7.8	3.0–4.9	4.9 (2.7–8.1)	2.8 (1.2–5.5)
284	<6.9	<3.0	3.9 (1.9–6.8)	2.1 (0.8–4.5)
Background population	–	–	2.8	0.75

§ 33 cases without serious events and with missing data on early HbA1c.
* Standard reference 4.4–6.4 (mean +/– 2 SD)

Conclusion: The risk of PM and CM in type 1 diabetic pregnancies tended to increase even with slightly raised pre-conceptional HbA1c-values. The risk for PM and CM was markedly increased at z-scores above 7 and 10 respectively.
Support: The Danish Diabetes Association

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Prevalence of gestational diabetes mellitus (GDM) among 5489 multi-ethnic pregnant women in Montreal using a randomized trial of a 75 vs 100 g glucose load

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Intro: Universal screening for GDM at our institution is done using a 1 h 50g glucose screen (GS) between 24 and 28 weeks. If the GS plasma glucose (PG) is ≥10.3 mmol/L, GDM is diagnosed; and if between 7.8 and 10.3, an oral glucose tolerance test (OGTT) is required for diagnosis. Since 1998, the Canadian Diabetes Association (CDA) recommends either a 2 h 75g [fasting 5.3, 1 h 10.6, 2 h 8.9] or a 3 h 100 g OGTT using National Diabetes Data Group (NDDG) 1979 criteria [fasting 5.8, 1 h 10.6, 2 h 9.2, 3 h 8.9]. For either, GDM is diagnosed if = 2 values are met or exceeded; impaired glucose tolerance (IGT) is diagnosed if 1 value is abnormal.
Aim: In order to validate the CDA diagnostic criteria for a 75 g OGTT, we undertook a randomized control trial between January 2001 and July 2004, randomizing 5489 women into one of three CDA-recommended diagnostic methods. G1(n=1812) – 50 g GS and 100g OGTT, NDDG criteria; G2 (n = 1839) – 50 g GS and 75g OGTT, CDA criteria; G3 (n= 1838) – all had 75 g OGTT, CDA criteria for diagnosis.
Results: All 3 methods detect similar rates of overall glucose intolerance (GDM+IGT), however the 100 g OGTT picked up significantly more cases of GDM. In group 1 (G1) and G2, the GS alone diagnosed half of GDM cases. The 75g OGTT, in contrast, detects a higher proportion of IGT whether it=s used in a 1-step or 2-step strategy. GDM diagnosis by OGTT using the values recommended by ADA for 75g and 100g would have statistically different rates. Both CDA and ADA criteria give lower diagnostic rates than if WHO criteria were applied to the 75 g OGTT sample as seen in the Table:

Prevalence (%) of GDM and IGT by study group using various international criteria

Diagnostic Criteria	CDA 1	ADA 1	WHO 1	CDA 2	ADA 2	WHO 2	CDA 3	ADA 3	WHO 3
GDM by GS alone	2.2	2.2	2.2	2.6	2.6	2.6	–	n/a	N/A
GDM by OGTT	2.5	3.9	2.5	1.4*	2.4*	6.7*	2.9	4.4	13.2*
Total GDM	4.7	6.1	4.7	3.9*	5.0	9.3*	2.9	4.4*	13.2*
IGT	3.4	5.2	3.4	4.0	4.8	N/A	5.4	6.3*	N/A
GDM and IGT	8.1	11.3	8.1	7.9	9.8	9.3	8.4	10.6	13.2*

** Difference from G1 gold standard is significant (p<0.05) by chi-squared test.*

The glucose value most frequently abnormal and diagnostic was the 1 h PG for both glucose loads – abnormal G1 [fasting 2%, **1 h 75%**, 2 h 44%, 3 h 35%], G2 [fasting 16%, **1 h 67%**, 2 h 48%] and G3[fasting 28%, **1 h 59%**, 2 h 56%]. Using the 75g OGTT glucose values of G3, we derived normative values by removing women with fasting PG ≥ 5.8 or any value ≥ 11.1 mmol/L, adjusting the mean +2SD and weighting this value for variation attributable to ethnic diversity. The values obtained were: fasting 4.7, 1 h 10.3 and 2 h 8.7. When these values were applied to G2, the rates of GDM became very comparable to the original NDDG criteria used in G1 [i.e. GDM by GS alone 2.6%, GDM by OGTT 2.5%, GDM total 5.1%, IGT by OGTT 4.7%, and GDM + IGT 9,8%].
Conclusions: The criteria presently used for the CDA and ADA 75 g OGTT underdiagnose GDM compared to the 1979 NDDG criteria previously used. A GS above 10.3 mmol/L picks up almost 1/2 of the GDM population. The 1 h PG on a OGTT is the most diagnostic. To obtain comparable prevalence rates to the NDDG criteria, the 75g glucose values used for diagnosis would be: **fasting PG ≥ 4.7 mmol/L, 1 hPG ≥ 10.3 and a 2 h PG ≥ 8.7** as derived from normative values in 1838 multiethnic Montreal women.
Support: Canadian Diabetes Association

OP 10
Mitochondrial metabolism

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PGC-1α-responsive-genes for mitochondrial oxidative phosphorylation are up-regulated in liver of patients with type 2 diabetes

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Background and Aims: Oxidative phosphorylation (OXPHOS) plays a role in insulin sensitivity in skeletal muscle, and its activity is reduced in insulin-resistant offspring of type 2 diabetic patients. Peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α)-responsive genes involved in OXPHOS are coordinately down-regulated in skeletal muscle of type 2 diabetic patients. Since PGC-1α is known to play a central role in controlling hepatic gluconeogenesis and to be strongly induced in diabetic model mice, status of PGC-1α-responsive OXPHOS pathway in diabetic liver is of great interest. We comprehensively analyzed expression of PGC-1α-responsive OXPHOS genes in liver of type 2 diabetic (DM) patients by using serial analysis of gene expression (SAGE) technology.
Materials and Methods: Liver samples were obtained from DM patients (n=5, Male 3, Age 60 ± 11 yrs, BMI 25.2 ± 5.2 kg/m^2, FPG 171 ± 54 mg/dl, HbA_{1c} 8.1 ± 2.0%, Values are mean±SD) and five normal glucose tolerance (NGT) patients who had undergone surgical treatment for malignancy. Informed consent was obtained from all patients. SAGE libraries were constructed from 2.5 μg of RNA from each sample, and sequenced at random. The gene expression profiles of DM and NGT libraries were compared through the normalization to a total of 1,000,000 tags, and statistical significance between libraries was calculated with SAGE 2000 software.
Results: (1) A total of 144,901 sequence tags were obtained from the two libraries (DM 44280 tags, NGT 100621 tags). (2) The top class represented gene ontology cellular component for the DM library was "mitochondria," including genes coding 12S rRNA, 16S rRNA, cytochrome c oxidase subunit III, and ATP synthase F0 subunit 6, whereas that for the NGT library was "extracellular" including genes coding apolipoprotein C-I, apolipoprotein C-II and albumin. (3) Regulatory genes on hepatic gluconeogenesis encoding PGC-1α, TRB-3, mitochondrial phosphoenolpyruvate carboxykinase 2 (PEPCK2) and GLUT2 were significantly up-regulated in the DM library ($p<0.001$). (4) Fifty-three OXPHOS genes were found in the two libraries, and the overall expression level was 1.7 times higher in the DM library than in the NGT library (2355 vs. 1385 tags, $p<0.00001$). Thirty-six (68%) of the 53 OXPHOS genes were up-regulated in the DM library. (5) In addition to OXPHOS genes, the expression of mitochondrial 16S and 12S ribosomal RNA (rRNA) was up-regulated in the DM library, suggesting increased translational activity and enhanced intra-mitochondrial ATP production probably through the mitochondrial oxidative phosphorylation in type 2 diabetic liver.
Conclusion: Our comprehensive analysis revealed up-regulation of genes encoding PGC-1α-responsive OXPHOS and mitochondrial rRNA in liver of type 2 diabetes, and clearly support the concept that PGC-1α is a key modulator of hepatic gluconeogenesis in human type 2 diabetes. Regulation of PGC-1αin liver looks like a mirror image of that in skeletal muscle, in which the expression of PGC-1α and OXPHOS genes is reduced in type 2 diabetes. Tissue-specific regulation of PGC-1α in liver and muscle should be taken into account when considering therapeutic targets for hyperglycemia in type 2 diabetes. Searching for the upstream master gene that regulates tissue-specific expression of PGC-1α-responsive genes may become an important issue to elucidate the etiology of type 2 diabetes.

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ERRalpha is essential for mitochondrial function in skeletal muscle

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Background and aims: Levels of PPAR Gamma Coactivator -α (PGC-1α) responsive genes are known to be decreased in type 2 diabetics. The orphan nuclear receptor Estrogen-Related Receptorα (ERRα) has been shown to cooperate closely with PGC-1α in controlling the expression of genes critical in mitochondrial function. To further investigate the role of ERRα in PGC-1α-mediated mitochondriogenesis, we obtained a mouse strain with a targeted deletion in the DNA binding domain of the ERRα gene locus. We examined the effects of overexpression of PGC-1α in wildtype and ERRα "knockout" (ERRαKO) mouse embryonic fibroblasts (MEFs). Induction of mitochondrial oxidative phosphorylation (OXPHOS) and fatty acid oxidation (FAO) gene expression as well as FAO and citrate synthase enzymatic activities by PGC-1α were determined in the presence and absence of ERRα. Recent data indicate that PGC-1α induces genes involved in the protection from oxidative stress. We also investigated whether this effect is mediated through ERRα by measuring the levels of these genes in PGC-1α-expressing ERRαKO MEFs. We further validated the reduction in OXPHOS and FAO gene expression in muscle of ERRαKO mice.
Materials and methods: We isolated MEFs from embryos of ERRαKO mice. PGC-1α was overexpressed in these cells using adenovirus. These cells were tested for 1. gene expression 2. cytochrome C protein levels 3. fatty acid oxidation 4. citrate synthase activity. Further, we examined the levels of genes of fatty acid oxidation and oxidative phosphorylation in both gastrocnemius muscle and white adipose tissues of control and ERRαKO mice.
Results: Increases in expression of cytochrome C, ubiquinol-cytochrome C reductase-binding protein (UQCRB) (genes in the OXPHOS pathway) and CPT-1B (carnitine palmitoyl transferase-1B, a rate-limiting step in FAO) were observed in response to PGC-1α expression in control MEFs, but were absent in ERRαKO MEFs. This translated into a lack of a significant increase in levels of cytochrome C protein expressed in these cells in response to PGC-1α, as compared to control MEFs which showed a 37% increase in cytochrome C protein. Increase in FAO (32% increase from basal) and citrate synthase (119% elevated from basal) activities induced by PGC-1α in control MEFs, did not occur in the ERRαKO cells. Similarly, increases in levels of oxidative stress protectant genes such as superoxide dismutase (6 fold), peroxiredoxin 5 (1.5 fold) and thioredoxin 2 (2 fold) in response to PGC-1α, were absent in these cells. Expression of OXPHOS (Cytochrome C, cytochrome C oxidase IV, uncoupling protein 3) and FAO (CPT-1B, medium-chain acyl CoA dehydrogenase, MCAD) genes were also reduced by approximately 50% in gastrocnemius muscle of ERRαKO mice.
Conclusions: ERRα is necessary for PGC-1α mediated increases in mitochondrial function. This is demonstrated at the level of changes in gene expression as well as mitochondrial function, as evidenced by cytochrome C protein levels, fatty acid oxidation and citrate synthase activity in the ERRαKO fibroblasts. This concept is further validated in vivo in muscle from ERRαKO mice that have a reduced complement of genes of mitochondrial function. We have established that genes for oxidative stress protection are ERRα-dependent. Thus, ERRα plays a central role in the control of PGC-1α-mediated pathways in mitochondria.

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Mitochondrial dysfunction caused a metabolic disorder showing the characteristics of type 2 diabetes

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Background and Aims: Insulin resistance, obesity and type 2 diabetes are the most serious threats to human health. However, the molecular mechanisms underlying these disorders and their interrelations are not well understood. In an effort to identify unknown causes of type 2 diabetes, we investigated whether mitochondrial dysfunction cause the development of insulin resistance and diabetes especially in skeletal muscles, one of the key insulin-responsive organs responsible for maintaining glucose homeostasis
Materials and Methods: Cells containing functionally-inactivated mitochondria were obtained by three different ways; disturbance of the mitochondrial electron transport with a treatment of inhibitors for 24 hours to the murine C2C12 myoblasts, and selective depletion of mitochondrial DNAs (mtDNAs) from the C2C12 cells either by incubating the cells in a medium containing low concentrations of EtBr (200 ng/ml) for 4 weeks (ρ o cells) or by transfecting them with si-mtTFA. The treated cells were collected and used for the isolation of total protein and RNA. Changes of target molecules related to the insulin signaling and to the glucose metabolism were analyzed mainly by Western blot and RT-PCR. Effects of mitochondrial dysfunction on glucose utilization were investigated by measuring the Glut4 translocation to plasma membrane and the uptake of [^{3}H]-2-deoxy-glucose into the cells.
Results: Mitochondrial membrane potential was decreased and intracellular Ca^{2+} level was dramatically increased, when mitochondrial function was reduced. Expression of IRS-1, an early insulin signaling molecule, was also reduced by mitochondrial dysfunction. The reduced IRS-1 expression was recovered either by removing the mitochondrial inhibitors or by chelating the intracellular Ca^{2+}. Furthermore, the activities of protein kinases related to obesity and insulin resistance, e. g., such as JNK and p38 MAPK, were elevated, resulting in the partial reduction of IRS-1 expression and of IRS-1 inactivation by Ser phosphorylation. As a consequence, overall insulin signaling was reduced. Finally, Glut4 expression and its translocation to the

plasma membrane were reduced by mitochondrial dysfunction, leading to the decrease of glucose uptake into C2C12 cells. All the results indicate the abnormal utilization of glucose as observed in type 2 diabetes.
Conclusion: Mitochondrial dysfunction caused a metabolic disorder like type 2 diabetes by affecting nuclear functions. As a result, the expressions of some genes closely related to insulin signaling and glucose metabolism were reduced. In turn, the affected overall genetic and cellular functions induced the aberrant insulin signaling and glucose utilization as observed in type 2 diabetes.
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Mitochondrial dysfunction in type 2 diabetes in relation to overweight and insulin resistance
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Background and aims: Insulin stimulates mitochondrial protein synthesis, enzyme activities and ATP production in lean insulin sensitive humans. Recently, evidence has been provided that nondiabetic but insulin resistant first-degree relatives of patients with type 2 diabetes mellitus (T2DM) exhibit mitochondrial dysfunction in skeletal muscle. However, no data are available on the relationships between body fat mass, insulin sensitivity and mitochondrial function in patients with overt T2DM. Thus, the present study assessed mitochondrial function from ATP synthesis in well-controlled T2DM during fasting and insulin stimulation.
Materials and Methodes: Six T2DM (4 m/2 f; age: 59 ± 3 a; BMI: 25.4 ± 1 kg/m^2; HbA1c: 6.6 ± 0.3%) were studied during fasting and hyperinsulinemic (40 mU · m^{-2} · min^{-1})-euglycemic (~100 mg/dl) conditions after 12 h overnight fasting. None had been treated with insulin before, oral hypoglycemic drugs exept for glitazones were allowed and withdrawn at least 3 days before the experiment. Insulin sensitivity was assessed from the M value over the last 30 min of the clamp test. Flux through ATP synthase was determined during fasting and clamp conditions with magnetization saturation transfer experiments using in vivo ^{31}P magnetic resonance spectroscopy of the calf muscle.
Results: Fasting plasma glucose concentrations (FPG) were 144 ± 5 mg/dl and mean clamp plasma glucose was 103 ± 1 mg/dl. Muscle ATP synthesis was 6.47 ± 0.8 µmol/g muscle/min during fasting and 7.98 ± 0.80 µmol/g muscle/min during the last 2 h of the clamp (p=0.118 vs. fasting). Correlation analysis did not reveal a relationship between FPG or HbA1c on basal (fasting) and insulin stimulated ATP synthesis, whereas BMI correlated inversely with basal ATP synthesis (r=–0.947, p=0.014) after adjustment for insulin sensitivity (mean M value: 6.23 ± 0.78 mg · kg^{-1} min^{-1}). The relationship between BMI and clamp ATP synthesis did not achieve statistical significance (r=–0.771, p=0.072). Insulin sensitivity positively correlated with clamp ATP synthesis (r=0.886, p=0.019) but not with basal ATP synthesis.
Conclusion: These results suggest that in T2DM even a small degree of overweight is related to reduction in mitochondrial function. Despite near-normoglycemic glycemic control, insulin resistance seems to impair also insulin stimulation of ATP synthesis which could relate to both genetic and nutritive factors.
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Modification in UCP3 and PGC-1 after surgically body weight loss
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Background and Aims: The aim of the study was to investigate the modification accompanying surgical body weight loss and particularly the change in muscular proteins involved in lipid oxidation.
Materials and Methods: Eighteen morbidly obese women were investigated before, 3 and 12 months after Roux-en-Y bypass, a restrictive surgical procedure, leading to a severe caloric restriction.
The group included 9 normoglycemic(NG) and 9 diabetic (DM) patients and we measured the following parameters: Energy Expenditure and substrate oxidation rate (indirect calorimetry); mRNA expression of UCP3 and PGC-1 in muscle biopsies (RT-PCR) and insulin sensitivity (euglycemic hyperinsulinemic clamp).
Results: The mean weight loss in the NG and DM patients was similar (mean:– 41+/–2 kg). 50% of the total body weight lost occurred between 0 and 3 months(rapid phase) and 50% between 3 and 12 months post-surgery (slow phase). No difference was observed between the 2 groups. As expected, insulin sensitivity pre-operatively was significantly different (p 0.001) and leads to normal blood glucose values in both group. Lipid oxidation increased significantly at 3 and 12 months in the NG group (p<0,05), but not in the diabetic one. Circulating free fatty acids (FFA) remained stable in both groups and weren't affected by weight loss. UCP3 values measured before surgery in both groups (NG: 0.51+/–.08 and DM: 0.68+/–0.17) were similar and correlated positively with FFA values (p<0.05). UCP3 values weren't modified after the rapid phase body weight loss but surprisingly UCP3 mRNA decreased at 12 months only in the initially NG patients (p<0,05). FFA values didn't correlate any longer to UCP3 at 3 and 12 months.PGC-1 mRNA values increased significantly after body weight loss in both groups respectively (p<0,05, before surgery: NG:0.994+/–0.33 vs DM:0.869+/–0.17; 12 months: NG 1.516+/–0.44 vs DM: 1.452+/–0.34). We also observed a correlation between the difference in PGC-1 and blood insulin concentration at 3 months and 12 months (p<0.05).
Conclusion: This study is the first to show an increase in muscle PGC-1 mRNA after surgically body weight loss. This increase in PGC-1 could be a contributing factor to the better insulin sensitivity observed at 3 and 12 months as previously shown in animal studies. In contrast UCP3 mRNA doesn't seem to be modified by body weight loss except at 12 months and only for the ND group. Further studies will permit to better understand if these modifications contribute to explain why the DM group can't increase lipid oxidation at 12 months and to better explain the relation between insulin concentration and PGC-1 mRNA in muscle.
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Mitochondrial fat metabolism and genes in human muscle are down-regulated by detraining prior to changes in insulin sensitivity
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Background and Aims: Insulin resistance is associated with muscle mitochondrial dysfunction and alterations in fat metabolism. The cause and effect relationship and the involvement of physical inactivity in this association are unknown. Fourteen healthy trained men underwent a 4-week detraining period to study the early changes in muscle mitochondrial fat oxidative capacity (OX_{FA}), intramyocellular lipid content (IMCL) and insulin sensitivity.
Materials and Methods: Muscle fat oxidative pathway (biochemical assay and mRNA expression level), plasma substrate concentration, respiratory exchange ratio (RER) during rest and exercise (indirect calorimetry), IMCL (^{1}H-MRS), and insulin sensitivity (3 h-insulin clamp), were measured before and after detraining.
Results: PPARα mRNA expression level was reduced by 58% (P<0.001) and PGC1α and adiponectin receptor 1 gene expression by 30–35% (P<0.01) in association with significant decrease in 1) plasma fatty acid concentration, 2) OX_{FA} and mitochondrial fat-oxidative enzyme activity, 3) mRNA expression level of proteins involved in fatty acid uptake, activation, transport, and oxidation, and significant increase in RER during exercise at moderate intensities (25–60%$VO_{2\,max}$). By contrast, complex I and cytochrome c oxydase activities as well as IMCL and insulin sensitivity were not significantly altered.
Conclusion: Short-term detraining coordinately down-regulated muscle mRNA expression of PPARα, PGC1α, adipoR1 and fat oxidative metabolism. Furthermore, OX_{FA} impairment is an early metabolic event that precedes the deterioration of insulin sensitivity.
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OP 11
Beta cell signal transduction

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Acute knockout of glutamate dehydrogenase in beta-cells obtained from transgenic mice profoundly modifies secretory responses

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Background and Aims: Glutamate dehydrogenase (GDH) is a mitochondrial matrix enzyme using NADH as cofactor and controlling the reversible reaction between alpha-ketoglutarate and glutamate. GDH is well recognized for its important contribution in the control of insulin secretion in beta-cells. However, the specific pathways and molecular mechanisms implicated are still a matter of debate. GDH is encoded by a well-conserved 45 kb gene, namely Glud1, organised into 13 exons. Previously, the NADH binding site of GDH was identified in residues Cys-270 through Lys-289. At the genomic level, we localised this essential binding site on exon 7 of Glud1. We recently generated transgenic mice carrying Glud1 exon 7 flanked by lox/P sites, thereby enabling conditional tissue-specific knockout. In the present study, GDH was abrogated acutely in beta-cells of islets isolated from floxed mice.
Materials and Methods: As a global strategy, we avoided abrogation of GDH in every tissue, with potential lethal effects, by use of the lox/P-flp-recombinase technology in order to target GDH knockout to tissues of interest, e. g. in beta-cells. It was achieved by generating F1 mice carrying mutant Glud-1 containing exon 7 flanked by lox/P sites following homologous recombination in embryonic stem cells. Using F1 mice, we performed acute in vitro GDH knockout on isolated islets transduced with an adenovirus encoding the Cre recombinase under the control of the rat insulin promoter (RIP-Cre). This resulted in the knockout of GDH exon 7 specifically in beta-cells accompanied with expected abrogation of GDH activity. GDH exon 7 deletion was assessed by PCR reaction and secretory responses were measured over 30-min stimulation periods following culture in RPMI-1640 complete medium.
Results: Three days after transduction of isolated islets with the adenovirus RIP-Cre, deletion of the floxed Glud1 exon 7 mediated by beta-cell targeted expression of Cre recombinase was controlled by PCR. In control islets, we obtained the expected band of 828pb. In islets of transgenic animals, the band was shifted down to 278bp, thereby demonstrating efficient genomic recombination. GDH knockout did not change islet morphology and survival. Insulin release in control mouse islets was stimulated 13.9-fold at 22.8 mM glucose compared to basal 2.8 mM ($p<0.005$). Acute knockout of GDH did not modify basal insulin release at 2.8 mM glucose. However, GDH abrogation in beta-cells markedly potentiated the secretory response at 22.8 mM glucose by 220% compared to control islets ($p<0.05$). Six days after adenovirus transduction, similar patterns were observed.
Conclusion: In the present study, we took advantage of our recently generated mice with floxed Glud1 exon 7 for acute knockout of GDH in isolated islets. First results demonstrate marked potentiation of glucose-stimulated insulin secretion. Current investigations aim at the delineation of the mechanisms explaining this unexpected consequence of GDH abrogation in cultured islets.

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MafA regulates pancreatic beta-cell-specific gene expression and insulin secretion

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Background and Aims: It has been shown that the pancreatic beta-cell transcription factor MafA is a key regulator of insulin gene transcription in both islets and insulinoma cells. The present study is aimed at investigating the effects of MafA on insulin secretion and beta-cell gene expression in INS-1 cells.
Methods and Materials: We have employed the Tet-On system to establish two INS-1 stable cell lines permitting, respectively, overexpression or dominant-negative suppression of MafA. Two stable INS-1 clones called, respectively, MafA*22 and DN-MafA*39 were selected for the present study. Both MafA and DN-MafA were epitope-tagged with HA (hemagglutinin). DN-MafA contains intact DNA-binding domain but lacks transcriptional activity. It thus exerts its dominant-negative function by competing with endogenous MafA for the cognate DNA binding.
Results: Immunofluorescence staining with HA-antibody showed that both MafA and DN-MafA proteins were induced in a doxycycline-dependent and all-or-none manner. EMSA demonstrated that the MafA binding to the rat insulin promoter was enhanced by overexpression of MafA and diminished by induction of DN-MafA. Both insulin mRNA levels and cellular insulin content were increased by MafA and reduced by DN-MafA. Glucose-stimulated insulin secretion, expressed as percentage of insulin content, was facilitated by MafA and blunted by DN-MafA. This is partly due to up- and down-regulation of glucokinase expression, which confers beta-cell glucose sensing, rather than changes in cellular insulin content. In addition, the expression of many beta-cell-spesific genes such as IAPP, Glut2, Pdx-1, Nkx6.1, GLP-1R, prohormone convertase-1/3, and pyruvate carboxylase was regulated positively by MafA and negatively by DN-MafA. Unlike Pdx-1, MafA gene manipulation did not affect glucagon mRNA levels.
Conclusion: These data suggest that MafA is not only a key activator for insulin transcription but also a master regulator of many beta-cell-specific genes, implicated in maintaining beta-cell phenotype, metabolism-secretion coupling, proinsulin processing, and GLP-1-signaling.

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Extracellular matrix-induced NF-κB activity in pancreatic beta cells is involved in spreading, actin cytoskeleton remodelling and glucose-stimulated insulin secretion

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Background and Aims: Laminin-5, a major component of the extracellular matrix derived from 804G cells (804G-ECM) induces spreading of pancreatic beta cells, actin cytoskeleton remodelling and improves their function (glucose-stimulated insulin secretion, GSIS) and survival. Furthermore, 804G-ECM induces transient activation of the transcription factor NF-κB and overexpression of its target genes IκBα and NF-κB1 (p105). NF-κB was previously shown to mediate the deleterious effects of cytokines on the pancreatic beta cell. The aim of this work was to investigate the involvement of ECM-induced NF-κB activity in the positive effects of 804G-ECM on the beta cell.
Materials and Methods: All experiments were performed on FACS-sorted primary rat pancreatic beta cells plated on poly-L-lysine (pLL, control) or on 804G-ECM-coated dishes. NF-κB activity was inhibited using adenovirus expressing non-phosphorylable IκBα mutant (IκBαnp) or with a pharmacological inhibitor of IκBα phosphorylation (Bay 11-7082, 5 μM). Spreading of cells (area; arbitrary units (a.u)) was quantified using ScionImage™ software. Actin cytoskeleton was visualized using Alexa-Fluor® 546-Phalloidin and confocal microscopy. Cell death was analyzed by TUNEL and short-term insulin secretion secreted over 1 h at 2.8 or 16.7 mM glucose (expressed as percent of total insulin content) was measured by radioimmunoassay. Data are presented as mean ± SEM for "n" independent experiments and levels of significance for differences between groups were assessed by Student's t-test for unpaired groups.
Results: Treatment of cells with Bay 11-7082 or with IκBαnp-expressing adenovirus significantly reduced spreading of cells on ECM as assessed after 24 h of culture (ECM: control: area=0.12 ± 0.01; Bay 11-7082: area=0.07 ± 0.01, $p<0.02$, n=4). Basal insulin secretion after culture for 24 h was inhibited two-fold in cells treated with Bay 11-7082 (pLL: control 0.42 ± 0.04%, Bay 11-7082: 0.21 ± 0.01%, $p<0.002$, n=6; 804G-ECM: control: 0.47 ± 0.04%, Bay 11-7082:0.21 ± 0.03%, $p<0.001$, n=6). Furthermore, GSIS (16.7 mM Glucose) was inhibited 5-fold (804G-ECM control: 9.2 ± 1.3%, Bay 11-7082: 1.7 ± 0.4%, $p<0.0005$, n=5–6) or 3-fold (pLL control: 4.4 ± 0.06%, Bay 11-7082: 1.36 ± 0.25%, $p<0.0005$, n=5–6) in Bay 11-7082-treated cells compared to control. ECM-induced actin cytoskeleton remodelling was disrupted in cells treated with Bay 11-7082. By contrast, inhibition of ECM-induced NF-κB activity with Bay 11-7082 or with the IκBαnp adenovirus did not alter cell survival either on pLL or on 804G-ECM.
Conclusion: These results indicate that ECM-induced NF-κB activity is involved in the effects of 804G-ECM on cell spreading, cytoskeleton organization and glucose-stimulated insulin secretion, while it is not involved in 804G-ECM-induced cell survival. ECM-induced NF-κB activity is therefore beneficial for pancreatic beta cell function. This contrasts with cytokine-induced NF-κB activity that is pro-apoptotic and generally detrimental to beta cell function. We propose that differences in the kinetics and amplitude of NF-κB activation might explain these different functional outcomes mediated by NF-κB.

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Requirement of glucokinase and insulin receptor substrate-2 for compensatory beta-cell hyperplasia in response to high-fat-diet-induced insulin resistance

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Background and Aims: Glucokinase (Gck) plays a crucial role, as a glucose sensor, in the secretion of insulin from individual pancreatic β-cells. Heterozygous β-cell-type glucokinase knockout (*Gck*$^{+/-}$) mice showed impaired glucose tolerance due to decreased insulin secretion in response to glucose, on a standard chow. On a high-fat (HF) diet, wild-type mice showed marked β-cell hyperplasia, whereas *Gck*$^{+/-}$ mice failed to show such compensatory β-cell hyperplasia in association with decreased β-cell replication, despite the presence of a similar degree of insulin resistance. In this study, we investigated the molecular mechanisms for the impaired β-cell hyperplasia in *Gck*$^{+/-}$ mice on the HF diet.
Materials and Methods: We performed a DNA microarray analysis as a means of examining the gene expression profiles of the islets systematically. We confirmed the results with Western blot and RT-PCR analyses. We further investigated the role of Irs2 in the regulation of β-cell mass on the HF diet.
Results: Of the 12490 genes examined, 144 were overexpressed (by 2-fold or more) and 134 were underexpressed (by 2-fold or more) in islets from *Gck*$^{+/-}$ mice on the HF diet compared to the islets from wild-type mice on the HF diet. DNA chip analysis revealed decreased levels of expression of IGF-1-receptor (Igf1r) (2.4-fold) and insulin receptor substrate-2 (Irs2) (25-fold) in the islets of *Gck*$^{+/-}$ mice on the HF diet, compared with the islets of wild-type mice on the HF diet. Western blot and RT-PCR analyses confirmed up-regulation of Igf1r and Irs2 expression in the islets of the wild-type mice on the HF diet, compared with wild-type mice fed standard chow, and their reduced expression in the islets of *Gck*$^{+/-}$ mice on the HF diet, compared with the islets of wild-type mice on the HF diet. After 10 weeks on the HF diet *Irs2*$^{+/-}$ mice exhibited increases in body weight, blood glucose, serum insulin, and insulin tolerance similar to those of wild-type mice after 10 weeks on the HF diet. *Irs2*$^{+/-}$ mice had a mean islet diameter and β-cell area similar to those of wild-type mice on the standard diet, but significantly smaller increases in these parameters than in the wild-type mice on the HF diet. Moreover, while mean islet diameter was significantly greater, by 14%, in wild-type mice after 5 weeks on the HF diet than in those on the standard diet, there was no such increase in *Irs2*$^{-/-}$ mice after 5 weeks on the HF diet compared to those on the standard diet. To directly test our hypothesis that reduction in Irs2 explains the impaired β-cell hyperplasia in *Gck*$^{+/-}$ mice on HF diet, we crossed the *Gck*$^{+/-}$ mice with β-cell Irs2 transgenic (βIrs2Tg) mice, which expressed a low level of Irs2 (~2-fold) in β-cells under the control of rat insulin promoter. After 4 weeks on the HF diet, while βIrs2Tg mice had glucose tolerance similar to wild-type mice, βIrs2Tg*Gck*$^{+/-}$ mice had significantly better glucose tolerance than *Gck*$^{+/-}$ mice, indicating that slight up-regulation of Irs2 in β-cells prevents the exacerbation of diabetes in *Gck*$^{+/-}$ mice.
Conclusion: Glucokinase and Irs2 are critical requirements for β-cell hyperplasia to occur in response to HF-diet-induced insulin resistance.

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Alterations of insulin signaling pathway in type 2 diabetic islets and the beneficial effects of metformin

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Background and Aims: In Type 2 diabetes, dysregulation of insulin signaling pathway (ISP) leads to reduced insulin action in muscle, liver and adipose tissues. In addition, ISP seems to be involved also in the regulation of pancreatic beta-cell mass and function.
Materials and Methods: We evaluated several steps of ISP in isolated human islets obtained from the pancreas of 6 non-diabetic (ND, age: 64.5 ± 9.7 yrs; gender: 4M/2F; BMI: 26.0 ± 4.4 Kg/m^2) and 5 Type 2 diabetic (T2DI, age: 60.5 ± 10.3 yrs; gender: 3M/2F; BMI: 25.8 ± 4.0 kg/m^2) multiorgan donors. Messenger RNA expression of insulin receptor (IR), insulin receptor substrate 1 and 2 (IRS1 and IRS2), and phosphatidylinositol 3-kinase (PI3K, p110 alpha subunit) was assessed by quantitative Real-Time (RT) PCR. In addition, PI3K, IRS1 and IRS2 protein expression and/or phosphorylation was evaluated by immunoblotting experiments.
Results: Compared to ND, T2DI showed a marked reduction of mRNA expression of IR (–80 ± 10%), IRS1 (–75 ± 13.2%), IRS2 (–67 ± 23%), and PI3K (–87 ± 5.8%) (all $p<0.01$). In addition, protein expression of PI3K was significantly ($p<0.05$) lower in diabetic islets (12.9 ± 7.2 optical density units, OD) than in control cells (21.5 ± 1.2 OD). Most importantly, the proportion of phosphorylated IRS1 and IRS2 protein was significantly ($p<0.05$) lower in diabetic (respectively 26 ± 11% and 54 ± 10%) than in non-diabetic (respectively 57 ± 22% and 73 ± 4%) islets. Interestingly, 24 h pre-exposure of Type 2 diabetic islets to therapeutic concentration of metformin determined a significant ($p<0.05$) increase of IR (+177 ± 39% vs untreated T2DI), IRS1 (+97 ± 36%), IRS2 (+85 ± 46%) and PI3K (+225 ± 65%) mRNA expression. In addition, PI3K protein expression in T2DI increased to 16.3 ± 1.9 OD, and IRS-1 and IRS-2 phosphorylated protein proportions increased to 83.7 ± 0.3% and 82.4 ± 1.2%, respectively (all $p<0.05$ vs untreated T2DI).
Conclusion: These results show that: 1) ISP is impaired at multiple levels in Type 2 diabetic islets, which may contribute to the defects of beta-cell function and survival in this disease; and 2) ISP alterations appear to be pharmacologically reversible. Therefore, improving insulin action in the beta-cells might represent an additional target of Type 2 diabetes therapy.

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The diabetes-linked transcription factor Pax4 is expressed in human pancreatic islets and is activated by glucose, activin A and betacellulin

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Background and Aims: Polymorphisms in the pax4 gene have been associated with Type 1 diabetes while point mutations have been linked to type 2 diabetes, implicating Pax4 in mature β-cell function and/or regeneration. We have recently demonstrated that induction of endogenous Pax4 gene expression coincides with β-cell proliferation induced by the mitogens activin A and betacellulin in adult rat islets. Consistent with a proliferative role of Pax4, we also demonstrated using recombinant adenoviruses, that rat and human βells overexpressing Pax4 displayed greater replication rates as compared to control-infected islets. To further substantiate our findings, we evaluated the impact of mitogens on endogenous Pax4 expression both at the transcript and protein level in human islets. Furthermore, in order to evaluate the direct contribution of Pax4 on β-cell plasticity, we have developed a RNA interference (RNAi) strategy to suppress Pax4 activation in response to mitogens.
Materials and Methods: Immunofluorescence studies were performed on partially trypsinized human islets for the transcription factors Pax4, Pdx1, Nkx6.1, Isl1, Ngn3 and insulin. Isolated human islets were exposed to either 5.5 or 11 mM glucose for 24 and 48 hours. Islets were also treated with 0.5 nM of activin A, betacellulin or TGF-β1 for 24 hours. Steady state mRNA levels for Pax4 were quantified by quantitative real-time RT-PCR and normalized to cyclophilin. A 21-nucleotide Pax4 hairpin RNA structures (siPax4) was cloned into the pDLDU6 vector and transfected into the rat insulinoma cell line INS1E along with GFP using lipofectamine. Subsequent to cell sorting using GFP (72 hours post-transfection), the effects of RNAi on endogenous Pax4 transcript levels were quantified by real time RT-PCR.
Results: We initially performed a comparative profile of transcription factors in human islets to establish relative expression levels of Pax4. Low but consistent levels of Pax4 mRNA and protein were detected in isolated human islets as compared to Nkx6.1, Pdx1 and Isl1. In contrast, Ngn3 was undetectable. In parallel, we found that exposure to 11 mM glucose for 48 hours resulted in a 3.6-fold increase in Pax4 mRNA levels as compared to control 5.5 mM glucose. Treatment with either 0.5 nM activin A or betacellulin for 24 hours resulted in a 3.5- and 8 fold increase in Pax4 transcript, respectively. In contrast, TGF-β1 was ineffective. These results provide evidence that Pax4 activity is regulated by physiological stimuli in human islets. We are currently examining the effect of mitogen-induced Pax4 activation on β-cell proliferation. The insulinoma INS1E cells expressed high levels of Pax4 mRNA. Pax4 steady state mRNA levels were lowered by 80% in INSIE cells co-expressing GFP and siPax4. Repression was specific since mRNA levels for PDX1 and insulin remained constant.
Conclusion: Taken together, these data suggest that Pax4 is induced by mitogens known to promote pancreatic islet cell proliferation. Expression of a Pax4 targeted siRNA reduced the transcription factor expression in INS1E cells, allowing us to address the direct implication of Pax4 in β-cell function in rat and human islets.

OP 12

Nephropathy, pathogenetic mechanisms

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TGFβ1-dependent renal fibrosis is negatively regulated by protein kinase C epsilon in experimental murine diabetic nephropathy

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Background and Aims: We have previously demonstrated that PKC-epsilon-expression was increased in the kidney from streptozotocin (STZ)-induced diabetic rats. In the present study, we tested the hypothesis that deletion of PKC-epsilon-expression in vivo will lead to protection against the development of murine experimental diabetic nephropathy.
Materials and Methods: We studied PKC-epsilon knock-out (KO) mice (n=11/5) compared to C57BL/6J wild type (WT) mice (n=9/8) in nondiabetic and diabetic condition. Hyperglycaemia was induced in 8 week old mice by intraperitoneal streptozotocin (STZ) injection(50 mg/kg/body weight on day 1 to 5). Diabetic mice (blood glucose > 20 mmol/l) remained hyperglycaemic (WT: 7.8 ± 0.9 vs. 20.3 ± 6.6 mmol/l and KO: 7.9 ± 1.1 vs. 22.1 ± 7.3 mmol/l). After 8 weeks animals were sacrificed and kidneys were removed.
Results: Body weight in the non-diabetic groups was higher due to glucosuria in the diabetic groups. Urinary albumin/creatinine ratio was significantly increased in PKC-epsilon-KO mice compared to WT mice (10.4 vs. 36.2 g/mol) which was further exacerbated in diabetic (PKC-epsilon-KO) mice (11.5 vs. 140.1g/mol) ($p<0.5$). Light microscopy showed more severe glomerular and tubulointerstitial fibrosis in diabetic PKC-epsilon KO compared to diabetic WT mice. Interestingly, the expected increased fibrosis in diabetic groups was already observed in PKC-epsilon KO mice under non-diabetic conditions while a significant increase of glomerular TGF-beta1 expression in PKC-epsilon KO compared to WT mice was further aggravated in the diabetic state. On the tubulointerstitial level, the expression of fibronectin and collagen IV as marker of renal fibrosis was also significantly increased in (diabetic) PKC-epsilon KO mice.
Conclusion: Our data suggest that PKC-epsilon expression is protective against renal fibrosis and that previously observed PKC-epsilon expression in the diabetic state may rather be a response-to-injury than a pathogenic effect itself.
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Serum collagenolytic activity, urinary hydroxyproline excretion and glomerular accumulation of type III, IV and VI collagens in early diabetic nephropathy in type 1 diabetes

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Background and Aims: The excessive accumulation of collagen is considered as a cornestone in development of diabetic glomerulosclerosis, but precise mechanisms and laboratory markers of collagen accumulation remain to be clarify. In this study we investigated serum collagenolytic activity (CLA), urinary hydroxyproline excretion and glomerular occurrence of type III, IV and VI collagens in diabetic nephropathy in type 1 diabetes.
Materials and Methods: 62 type 1 diabetic patients with normal creatinine clearance, 28 M/34 F, 30.6 ± 9.6 (SD) years, were examined. 20 patients were normoalbuminuric (group DN0), 24 ones had microalbuminuria (group DN1) and 18 patients had macroalbuminuria (group DN2). The total CLA and urinary excretion of free and peptide-bound hydroxyproline (fHP, PBHP) were measured and compared with control (29 healthy subjects, group C). Immunohistochemical staining of type III, IV and VI collagens was investigated in kidney biopsy specimens from 17 patients, including 11 normoalbuminuric, 4 microalbuminuric and 2 macroalbuminuric ones. The kidneys specimens from 10 healthy subjects were acted as control.
Results: Serum CLA was markedly higher in all diabetic groups as compared to control (DN0: 21.7 ± 14.6, DN1: 28.1 ± 21.1, DN2: 24.9 ± 20.2 vs. C: 10.5 ± 5.8 $\mu mol \times l^{-1} \times h^{-1}$, all $p<0.001$). Excretion of PBHP was increased in patients with micro- and macroalbuminuria (DN1: 5.9 ± 3.4, $p=0.001$, DN2: 6.6 ± 5.3, $p=0.004$, C: 3.4 ± 1.8 mg/mmol creatinine), but not in normoalbuminuric patients (3.7 ± 3.2, $p>0.05$). The increase in fHP excretion was not significant (DN0: 1.2 ± 1.2, DN1: 1.5 ± 1.1, DN2: 1.4 ± 0.9, C: 1.1 ± 0.9 mg/mmol creatinine, all $p>0.05$). There was a negative correlation between CLA and PBHP ($r=-0.49$, $p=0.02$). In a multiple regression analyses albumin excretion rate and glycemia were independently associated with CLA ($R^2=0.38$, $p=0.04$), meanwhile diabetes duration and HbA1c were associated with PBHP excretion ($R^2=0.33$, $p=0.01$). The excessive staining of type IV and type VI collagens in glomeruli was found in 8 cases (six DN0-patients, one DN1- and one DN2-patient) and in 7 cases (four DN0- and three DN1-patients) respectively. Besides, interstitial type III collagen was revealed in glomeruli in 9 cases (five DN0- and three DN1-patients, one DN2-patient), meanwhile it was negative in control. Patients with at least one collagen excessive staining (n=11) had greater 24-hour diastolic blood pressure as compared to other patients (79.8 ± 4.8 vs. 73.4 ± 0.9 mmHg, $p=0.006$), but no differences have been found in age, albuminuria, systolic blood pressure, HbA1c, glomerular filtration rate, CLA and HP.
Conclusion: The obtained data demonstrate the disturbances in the collagen metabolism, as well as accumulation of type IV and VI collagens and appearance of interstitial type III collagen in the renal glomeruli in Type 1 diabetes. These changes are present on the early stages of diabetic nephropathy and may play an important role in progression of glomerulosclerosis.

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TGF-β1 and mechanical stretch reduce murine podocyte adhesion to extracellular matrix substrate and modulate β1 integrin expression/ maturation *in vitro*

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Background and Aims: Alterations of renal haemodynamic and TGF-β1 activity play an important role in the pathogenesis of diabetic glomerulopathy. Podocyte adhesion to the glomerular basement membrane (GBM) is crucial in the maintenance of the anatomical and functional properties of the glomerular filtration barrier and their detachment from the GBM is believed to represent one of the mechanisms of proteinuria. We have previously shown that that high glucose and mechanical stretch (S) downregulate β1 integrin isoform, an adhesion molecule anchoring podocytes to GBM; and reduce podocyte adhesion to extracellular matrix substrates. β1 integrin isoform exists under a glycosylated/mature functional and non-glycosylated/immature non-functional form. In the present study, we investigated the role of TGF-β1 and S on the expression and maturation of β1 integrin in podocytes in vitro and examined the consequences on podocyte adhesion to extracellular matrix. We also studied the role of p38-MAPK, a signalling pathway induced in diabetic glomerulus on β1 integrin expression and maturation.
Materials and Methods: Conditionally immortalised murine podocytes (gift from Prof. P Mundel, New York – USA) were cultured on flexible silicone membrane plates coated with Human Extracellular Matrix. Podocytes were cultured in normal glucose (5.5 mM) and exposed to TGF-β1 (5ng/ml) for 3 to 48 h, or to S (20% elongation-1cycle/second) for 48 h. The interaction of S and TGF-β1 was examined by exposing cells to 48 h S followed by addition of TGF-β1 for 48 h. SB203580 (2 μM), a p38MAPK inhibitor, was added 1 h prior exposure to TGF-β1, S, or their combination. β1 integrin expression and maturation were assessed by western immunoblotting and cell adhesion on HECM by crystal violet assay.
Results: TGF-β1 downregulated the mature form of β1 integrin after 9 hours of exposure ($p<0.04$), and upregulated the immature form after 24 h ($p<0.002$). Stretch downregulated both the mature and immature forms of β1 integrin expression by ~23% ($p<0.0001$). TGF-β1 reversed the stretched-induced downregulation of the β1 immature form ($p<0.001$). Addition of SB203580 had no effects on stretched-induced changes but prevented the TGF-β1-mediated upregulation of β1 immature form ($p=0.028$). Similar results were obtained when cells were exposed to the combination of mechanical stretch and TGF-β1. TGF-β1 and S on their own reduced podocyte adhesion on HECM substrate by 10% and 24% respectively ($p<0.0001$).
Conclusion: Stretch-induced downregulation of both mature and immature forms of β1 integrin and TGF-β1 modulation of β1 integrin maturation, in favour of the immature from, are mechanisms likely to contribute to decrease podocyte adhesion to GBM and thus proteinuria. The TGF-β1-induced upregulation of β1 immature form is a p38 MAPK mediated event.
Support: Diabetes UK / NKRF

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Proteoglycan II plays a regulatory role in expression of TGF-beta1 and ECM in rat mesangial cells

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Background and Aims: There is increasing evidence indicating that mesangial autocrine activation of transforming growth factor-β1 (TGFβ1) mediates the effects of high glucose concentrations on kidney, and that TGFβ1 is a determinant in the development of human and experimental diabetic glomerulosclerosis. One of the main target cells for TGFβ1 in the kidney is glomerular mesangial cells. Disordered mesangial cell synthesis and catabolism of the components of the extracellular matrix (ECM) under high-glucose conditions are the immediate cause of this glomerulopahty. One of approaches which down-regulating TGFβ1 expressing is the use of the endogenous proteoglycanII (decorin), which is referred to as a natural inhibitor of TGFβ1. In the present study, we constructed recombinant adenovirus expressing rat decorin (Ad-decorin) and further investigated the effects of decorin overexpression on the expression of TGFβ1 and ECM in rat mesangial cells.
Materials and Methods: The recombinant decorin adenovirus and lacz adenovirus(Ad-lacz), as a control,were constructed.And MTT was used to examine the biological function of decorin (decorin expressed by Ad-decorin transduced CHO cells was used to interact with TGFβ1 which can inhibite the proliferation of CCL-64 cells). After that, Ad-decorin was used to transduced rat mesangial cells (RMCs) cultured in high-glucose (4500 mg/L) media and recombinant Ad-lacz as the control transducer. TGFβ1, decorin, collagen IV, fibronectin, lamnin and tenascin mRNA in RMCs from 24, 48, 72, 96 to 168 hours after Ad-decorin transduction were determined with RT-PCR. Cellular immunohistochemistry was used to dectect the distributing and expressing of TGFβ1 protein in RMCs at 96 hours after Ad-decorin transduction.
Results: MTT showed that decorin protein expressed by Ad-decorin transdued CHO cells abrogated the inhibitive the effect of TGFβ1 on CCL-64 cells. Decorin mRNA significantly increased in Ad-decorin transduced RMCs at every observed points, reached the peak at 24 hours (+231%, $P<0.01$) and the overexpression lasted to the end of the observation (+178%, $P<0.01$) compared to that in Ad-lacz transduced RMCs. Meanwhile,TGFβ1 mRNA level were prominently falled in Ad-decorin transduced RMCs from 72 hours (–45%, $P<0.01$) and was lowest at 168 hours (–60%, $P<0.01$). ECM components, such as tenascin (–54%, $P<0.01$), laminin (–57%, $P<0.01$), fibronectin (–59%, $P<0.01$) and collagen IV (–62%, $P<0.01$), were reduced notably in the Ad-decorin transduced RMCs from the 72 hours to the end of study versus those in the Ad-lacz transduced RMCs. Cellular immunohistochemistry further comfirmed that the Ad-decorin transduced RMCs produced much less TGFβ1 compared with the Ad-lacz transduced RMCs.
Conclusion: The constructed recombinant decorin adenovirus can efficiently express biologically active decorin. Overexpressing decorin can decrease the expression of TGFβ1 and ECM components of RMCs. These results suggest that overexpression of decorin may be one of efficiently theraputic approaches to diabetic nephropathy.
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Nephrin loss in experimental diabetic nephropathy is prevented by deletion of protein kinase C alpha signalling in-vivo

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Background and Aims: Albuminuria in diabetic nephropathy involves the disturbance of the fenestrated glomerular endothelium, the glomerular basement membrane as well as the epithelial slit diaphragm. We have recently demenstrated that protein kinase C alpha (PKCα) deficient mice are protected against the development of albuminuria under diabetic conditions probably due to a loss of the negatively charged heparan sulfate proteoglycans. We now analyzed nephrin protein and mRNA expression in these animals and found that the PKCα isoform is a negative regulator of nephrin mRNA expression under diabetic conditions.
Materials and Methods: Experiments were performed with male 129/SV PKCα$^{-/-}$ mice (KO) and 129/SV wild type (WT) animals. Hyperglycaemia was induced in 7 week old mice by intraperitoneal injection of streptozotocin (STZ) on day 1 and 4. After eight weeks of hyperglycaemia the non fasting blood glucose levels were 25.9 ± 3.4 mmol/l in the diabetic WT (n=6) and 32.2 ± 1.6 mmol/l in the PKCα$^{-/-}$ mice (n=7). In the sham-injected mice the levels were 12.2 ± 0.9 mmol/l in WT (n=7) and 10.9 ± 0.6 mmol/l in the PKCα$^{-/-}$ mice (n=7).
Results: After eight weeks of hyperglycaemia a significant higher urinary albumin excretion was detected in WT diabetic mice (24.78 ± 8.98 g/mol creatinine) compared with WT control mice (7.37 ± 0.47 g/mol creatinine) ($p<0.05$). Notably, no significant increase of the albumin/creatinine ratio was observed in the diabetic PKCα$^{-/-}$ (9.88 ± 1.67 g/mol creatinine) in comparison to control PKCα$^{-/-}$ mice (6.94 ± 1.81 g/mol creatinine). After sacrificing animals, the tissues were proceeded by immunohistochemistry, western blotting and real-time qPCR. Immunostaining revealed that the glomerular expression of nephrin was significantly reduced and barely detectable in diabetic condition. This diabetes-induced loss of glomerular nephrin expression was completely prevented in diabetic PKCα$^{-/-}$ mice. In WT diabetic animals the nephrin protein amount is reduced to a third of the values detectable in WT control mice. No reduction of the nephrin content is observed in diabetic PKCα$^{-/-}$ mice. Furthermore, we found a significant reduction of nephrin mRNA levels in whole kidney extracts from diabetic versus control WT mice. In contrast, no reduction of nephrin mRNA levels was observed in diabetic vs. control PKCα$^{-/-}$ mice.
Conclusion: We therefore suggest that an increased PKCα activity is of pivotal importance for the development of albuminuria in diabetes mellitus. The complex pathyophysiology may involve 1) down regulation of nephrin mRNA and protein expression with structural disturbance of the podocyte slit diaphragm as well as 2) changes in the glomerular basement membrane composition with loss of the negatively charged heparan sulfate proteoglycans.
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Identification of glomerular precursor cells in the adult kidney

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Background and Aims: It is well known from the clinical practice that renal tubuli are able to regenerate even after major damages. This property is not shared by other renal compartments such as the glomerulus. The consequence of this phenomenon is that renal pathologies, such as diabetic nephropathy, that are characterized by the loss of glomerular function invariably lead to an end stage renal failure. The identification of stem cells niches inside the glomerulus could allow to regenerate this renal component.
Material and Methods: With the aim to identify glomerular stem cells, we studied kidneys obtained from six C57BL/6 mice. Glomerular cores were isolated from the renal cortex by serial sieving, digested with collagenase and finally plated out using a specific culture medium. Glomerular cell outgrowths appeared after 7–10 days in culture. Cells were characterized by morphology and by specific antibodies (Thy1-1 for mesangial cells, cytokeratin for epithelial cells and Von Willebrand factor for endothelial cells).
Results: To verify the presence of progenitor cells inside the renal primary culture, immunohistochemistry for stem cell antigen-1 (Sca-1) was performed. As a result, about forty per cent of the colonies stained positive for this stem cells-related surface antigen. To further clarify the plasticity of glomeruli-derived cells, we tested the possibility, using a specific culture medium, to transform these cells into osteoblasts. The appearance of alkaline phosphatase activity on the membrane was used to confirm that the transformation was successful. As a result, after two weeks of treatment, about 35–40 percent of the colonies in primary culture contained cells expressing an active alkaline phosphatase on the surface. To further test the plasticity of these cells, glomerular-derived cells were co-cultured with myocardiocytes. After 4 days in culture about half of renal cells resulted positive for cardiomyosin, suggesting their transformation into myocardiocytes. Split after split, cultured cells had a tendency to differentiate and after 5 passages in culture only 2–3 per cent of cells stained positive for Sca-1.
Conclusion: In conclusion the glomerulus contains progenitor cells positive for Sca-1 and able to transform in vitro into osteoblasts and myocardiocytes. In vivo study are now needed to verify the possibility to repair glomerular damages using glomeruli-derived stem cells.

OP 13
Inhaled and oral insulin delivery

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Inhaled insulin (Exubera®) achieves tight glycemic control and is well tolerated in patients with type 1 diabetes

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Background and aims: Inhaled insulin (INH; Exubera®) is being investigated as an alternative, noninvasive method of insulin delivery. The effects of INH on glycemic control and pulmonary function were evaluated in patients with type 1 diabetes in a 24-week study.
Materials and methods: In this parallel-group, multicenter study, 226 patients with type 1 diabetes, aged 25 to 65 years, were randomized to either daily premeal INH or subcutaneous (SC) human insulin for 12 weeks (comparative phase), followed by 12 weeks of SC insulin (follow-up phase). End points included glycosylated hemoglobin (HbA_{1c}), hypoglycemia, standardized pulmonary function tests, insulin antibody levels, cough questionnaire data, and adverse events.
Results: Reported efficacy results are for the comparative phase. Baseline HbA_{1c} values were 7.5% for both INH and SC groups. By Week 12, declines from baseline HbA_{1c} were similar, resulting in final HbA_{1c} values of 7.1% and 7.0% for the INH and SC groups, respectively. During the comparative phase, overall hypoglycemic event rates were 6.8 and 5.5 events/subject-month, respectively; risk ratio 1.24; 90% confidence interval (CI), 1.17, 1.31. Severe hypoglycemia occurred in 9 INH patients and 17 SC patients, (risk ratio 0.52; 90% CI, 0.31, 0.87). Small treatment group differences in changes from baseline in lung function (forced expiratory volume in 1 second [FEV_1] and carbon monoxide diffusing capacity [DL_{co}]) occurred within 2 weeks of initiating INH therapy and resolved within 2 weeks of discontinuation. Mean change from baseline at Week 2 in FEV_1 and DL_{CO}, respectively, was –0.070 L and –0.973 mL/min/mm Hg for INH and –0.027 L and –0.246 mL/min/mm Hg for SC (adjusted difference FEV_1, –0.043 L; DL_{CO}, –0.727 mL/min/mm Hg). Antibody levels rose with INH therapy to a median of 37.0 µU/mL (mean, 134.3 µU/mL) by Week 12 and declined following drug discontinuation. Increased antibodies were not associated with any clinical findings. All-causality cough was reported in 30.9% and 7.8% of INH and SC patients, respectively. Cough generally was mild, not productive, and occurred within minutes of dosing. Overall adverse event profiles for both treatment arms were similar, and the majority of adverse events were mild to moderate.
Conclusion: INH (Exubera®) therapy over 3 months is well tolerated and as effective as SC short-acting insulin in achieving tight glycemic control in patients with type 1 diabetes.
Study was funded by Pfizer Inc and Aventis, a member of the sanofi-aventis Group

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Inhaled Technosphere/Insulin: Favourable time-action profile and low variability in comparison to subcutaneous human regular insulin

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Background and Aims: Timing and reproducibility and of insulin's metabolic effect is critical to achieve near-normal glucose control and to enable patients to make appropriate dose adjustments. We compared the time-action profiles and the intra-subject variability in insulin absorption and insulin effect between repeated doses of 48 IU inhaled Technosphere/Insulin (TI) and 24 IU sc injected human regular insulin (SC).
Material and Methods: TI and SC were given on 3 separate occasions each on separate study days in a randomized sequence in 12 insulin-treated subjects with Type 2 diabetes (10 males, 2 females, age 56 (range 40–65) years, diabetes duration 14.4 (3–29) years, HbA_{1c} 6.9 ± 0.9% (mean ± SD), all normal lung function (FVC, FEV_1 and VC ≥ 80% of predicted normal)). Using a euglycemic glucose clamp (clamp level 120 mg/dl), pharmacokinetic (PK) and pharmacodynamic (PD) time-action profiles were measured over 540 min following each form of insulin administration.Glucose infusion rates, as parameter of metabolic action, were adopted every minute by a Biostator, blood samples for PK assessments were taken at baseline and at 10, 20, 30, 45, 60, 75, 90, 105, 120, 150, 180, 240, 270, 300, 360, 420, 480, and 540 min after drug application. Variability of absorption and effect, expressed as CV% of AUC_{0-t}, was determined at 120, 180 and 540 min after dosing.
Results: TI showed a more rapid onset of action and higher peak insulin concentrations (INS) than SC (table, median INS-t_{max} 15 vs. 150 min, TI vs. SC, p<0.0001). TI reached maximal glucose infusion rate (GIR) values already at 79 ± 47 min, while the maximum effect of the SC dose occurred at 293 ± 83 min (p<0.0001). The AUCs for both INS and GIR curves were higher for TI compared to SC in the first two and three hours after administration (table). The variability in both insulin concentrations and insulin action was lower for TI compared to SC in the first 3 h after administration (table).

	Inhaled Technosphere Insulin		SC Human Regular Insulin	
	mean±SD	CV (%) [95% CI]	mean±SD	CV (%) [95% CI]
Pharmacodynamic (PD) Parameters, based on Glucose Infusion Rates (GIR)				
GIR-$AUC_{0-2\,h}$ (mg/kg)	265 ± 83 (44% of total)	23.4 [13.9–33.0]	211 ± 84 (16% of total)	39.2 [23.2–55.2]
GIR-$AUC_{0-3\,h}$ (mg/kg)	355 ± 119 (59% of total)	21.7 [12.9–30.6]	363 ± 153 (27% of total)	33.4 [19.8–47.1]
GIR_{max} (mg/kg/min)	4.5 ± 1.0+	22.0 [13.0–30.9]	5.5 ± 1.4	17.3 [10.3–24.4]
Pharmacokinetic (PK) Parameters, based on Plasma Insulin (INS) Concentrations				
INS-$AUC_{0-2\,h}$ (µU/ml)	6965 ± 2233* (56% of total)	19.1 [11.3–26.9]	5509 ± 1094 (24% of total)	27.1 [16.1–38.2]
INS-$AUC_{0-3\,h}$ (µU/ml)	8030 ± 2561 (64% of total)	18.2 [10.8–24.6]	8672 ± 1442 (38% of total)	25.0 [14.8–35.2]
INS-C_{max} (µU/ml)	124 ± 44+	20.4 [12.1–28.8]	63 ± 10	29.2 [17.3–41.2]

CI: Confidence Interval
* p<0.05 vs. SC, +p<0.0005 vs. SC (ANOVA, Mixed Effects Models)

Conclusion: Technosphere/Insulin shows a more rapid onset and a shorter duration of action than subcutaneous human regular insulin which should make it suitable for replacement of prandial insulin secretion in patients with Type 2 diabetes. In particular, TI should provide a lower risk of late post prandial hypoglycemia as, in contrast to SC, most of its glucose lowering effect occurs before the 3 hour point. Furthermore, the intra-patient variability of repeated inhalations of TI is superior to SC insulin during the first 3 hours after dosing which should facilitate dose titration.
MannKind Corporation initiated and financed this study

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The safety and efficacy of human insulin inhalation powder (HIIP) versus injectable insulin in patients with type 1 diabetes (T1D)

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Background and aims: Insulin delivery to the lung via inhalation is a promising alternative to the injectable route of insulin administration. The Lilly/Alkermes Inhaled Insulin System is designed to deliver consistent and reliable doses of HIIP using a simple, breath-actuated device. The present randomized, open-label crossover study was designed to compare the safety and efficacy of preprandial HIIP versus subcutaneously injected (SC) insulin (regular human insulin or insulin lispro) in patients with T1D.
Material and methods: One group received preprandial HIIP + insulin glargine once a day (QD), followed by SC insulin + insulin glargine QD. The other group received the reverse treatment sequence. Both treatment periods lasted for 12 weeks. The primary efficacy analysis was a noninferiority comparison of the mean difference in A1C between treatments, based on a one-sided 95% confidence interval (CI) using least squares mean (LSM) with an upper limit <0.3%. Secondary efficacy analyses included fasting blood glucose (FBG) monitored by the patient prior to the start of the morning meal. Safety assessments included DL_{CO} and hypoglycemia rates.
Results: Patients with T1D were 53% female with a mean age of 39 ± 12 years. Mean baseline A1C was 8.1% with little change during study (see Table), and mean baseline DL_{CO} was 26.9 mL/min/1 mmHg. DL_{CO} was sig-

nificantly decreased from baseline in the HIIP group compared with the SC insulin group. The decrease in DL_{CO}, however, was transitory and appeared to be reversible during the crossover period when patients received SC insulin. Rates for any hypoglycemia and severe hypoglycemia were similar between treatments. Better FBG levels with HIIP were associated with increased nocturnal hypoglycemia, which was managed with appropriate dietary recommendations and adjustments in dosing regimen.

Select Study Parameters at Endpoint (All Randomized Patients, Combined Periods)

Parameter	HIIP (N=133)	SC (N=126)	Difference	p-value
A1C (%)	8.0 (0.1)	8.1 (0.1)	–0.1	0.17
FBG (mg/dL)‡	146 (6)	163 (6)	–17	0.01
DL_{CO} (mL/min/1 mmHg)	25.3 (0.7)	26.3 (0.7)	–1.0	<0.001
Any hypoglycemia§	8.9 (0.7)	8.2 (0.8)	0.1	0.29
Severe hypoglycemia§	0.17 (0.06)	0.13 (0.06)	0.04	0.50
Nocturnal hypoglycemia§	4.2 (0.4)	2.7 (0.4)	1.5	<0.001

Values are LSM (SE)
95% CI upper bound = 0.02%
‡ Morning preprandial value; based on patient blood glucose monitoring
§ Rate adjusted for 30 days

Conclusion: Based on A1C values, the HIIP and SC insulin treatments were equivalent in efficacy. Although DL_{CO} was significantly decreased from baseline in the HIIP group compared with the SC insulin group, the change was small and not clinically meaningful for the 12 weeks of exposure to HIIP. The greater incidence of nocturnal hypoglycemia in the HIIP group versus the SC insulin group, together with better FBG in the HIIP group, suggests that future dosing regimens may require minor adjustments. Overall safety profile was similar between the HIIP and SC insulin groups.

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Onset of action of inhaled human insulin via the AERx® iDMS compared to subcutaneous human insulin and subcutaneous insulin aspart

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Background and Aims: New modes of insulin treatment are being developed in order to obtain near physiological insulin levels and increase compliance. AERx®insulin Diabetes Management System (AERx®iDMS) is developed for pulmonary administration of human insulin, providing meal-related insulin dosing. The aim of this single-centre, open-label, three-period cross-over trial was to compare the onset of action and duration of action of inhaled fast-acting human insulin via the AERx®iDMS to that of subcutaneously (s.c.) administered rapid-acting insulin analogue, insulin aspart, and s.c. fast-acting human insulin.
Materials and Methods: The trial included a total of 15 non-smoking subjects with type 1 diabetes (age 34 ± 10 years (mean ±SD), BMI 24.3 ± 2.3 kg/m^2, duration of diabetes 18.6 ± 9.9 years) who were randomised to receive a dose (0.3 AERx units/kg, U/kg or IU/kg) of inhaled insulin (AERx), s.c. insulin aspart (IA) and s.c. fast-acting human insulin (HI) respectively on three different dosing days in randomised order. The trial was carried out by means of 10-hour isoglycaemic glucose clamp (clamp level of 7.2 mM) and the glucose infusion rate (GIR) was recorded for 10 hours post dosing.
Results: The onset of action (defined as time to 10% of $AUC_{GIR(0-10\,h)}$) was faster for AERx (72 [62;82] min (Least Square Means (LSMeans)[95%CI]) compared to HI (89 [79;100] min, P=0.009), and not different from IA (66 [56;77] min, NS). Duration of action (defined as time interval from $t_{10\%AUC\text{-}GIR(0-10\,h)}$ to $t_{90\%AUCGIR(0-10\,h)}$) for AERx (291 [264;318] min) was not different to that of HI (297 [270;323] min, NS), but was longer than for IA (209 [182;235] min, P<0.001). The time to maximum GIR (t_{GIRmax}) for AERx (142 [106;178] min) was faster than HI (202 [167;237] min, P=0.01), while t_{GIRmax} for AERx did not differ to that of IA (136 [101;171] min, NS). There was no difference between the area under the glucose infusion rate curve ($AUC_{GIR(0-10\,h)}$) for the three treatments (AERx: 1971 [1575;2466] mg/kg, HI:1949 [1562;2431] mg/kg, IA: 2126 [1704;2652] mg/kg, P=0.68). No safety issues were raised in this trial; adverse events were few and mild.
Conclusion: The onset of action of inhaled fast-acting human insulin via the AERx®iDMS is not significantly different from s.c. insulin aspart, but is significantly faster than s.c. fast-acting human insulin, while the duration of action is not significantly different from s.c. fast-acting human insulin, but is significantly longer than s.c. insulin aspart. These characteristics make inhaled fast-acting human insulin via the AERx®iDMS suitable as a meal-related insulin.
Support: Novo Nordisk A/S

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Reduction of postprandial blood glucose excursions by an optimised formulation of oral insulin

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Background and Aims: Oral Insulin (OI) formulated with Emisphere's Eligen technology has been shown to exhibit a blood glucose lowering effect with a rapid onset of action. This makes OI attractive for prandial insulin therapy, however, the effect of OI is blunted when administered together with food. The aim of this pilot study was to identify an insulin/carrier-ratio optimised for pre-prandial administration of OI.
Materials and Methods: In a first subset of experiments, eight subjects with diet-treated Type 2 diabetes (age 56 ± 6 years (mean±SD), BMI 29 ± 4 kg/m_, HbA_{1c} 6.7 ± 0.7%) received single-dose administrations of two different formulations of OI (formulation A: 4 tablets, each 75 IU insulin + 100 mg carrier, formulation B: 2 tablets, each 150 IU insulin + 80 mg carrier). Both formulations led to a substantial decrease of fasting blood glucose, but formulation B had a stronger metabolic effect (maximum BG decrease –26 ± 21 vs. –37 ± 27 mg/dl). Therefore, formulation B was used in a second series of experiments performed in a single-blind cross-over design in a subgroup of 4 patients. In these subjects, formulation B was administered together with a mixed meal (441 kcal, 66% carbohydrates). Blood glucose and insulin excursions were followed for 4 hours and compared with the results obtained with a control formulation which consisted of the carrier only.
Results: In comparison to the control formulation insulin concentrations rose faster with OI with significantly higher values early after drug administration (table). In accordance, blood glucose was lower with OI from 50 min onwards (table).

	Blood Glucose Concentrations (mg/dl)		Plasma Insulin Concentrations (µU/ml)	
Time after meal ingestion (min)	Oral Insulin	Control (Carrier alone)	Oral Insulin	Control (Carrier alone)
Baseline	118 (21)	118 (22)	20.0 (11.2)	21.2 (19.1)
5	119 (23)	120 (24)	30.2* (13.4)	14.0 (7.2)
15	127 (23)	120 (24)	50.2* (23.8)	24.3 (13.8)
20	135 (36)	124 (27)	54.7* (19.6)	30.5 (14.7)
25	143 (33)	130 (27)	55.0+ (19.5)	27.7 (13.1)
40	163 (35)	159 (36)	64.9 (59.9)	46.1 (29.7)
50	158 (47)	175 (40)	81.6 (71.6)	57.9 (44.4)
60	173 (26)	182 (39)	87.1 (91.5)	73.3 (60.6)
120	147 (27)	169 (33)	79.9 (77.4)	72.8 (55.4)
240	103 (11)	119 (16)	36.7 (28.8)	27.8 (23.4)

Table gives mean (SD) values, *p<0.1 vs. control, +p<0.05 vs. control

Conclusion: These promising effects of oral insulin on postprandial blood glucose and insulin excursions warrant further investigation of this optimised oral insulin formulation in larger trials.
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A 12-day comparison of preprandial Humulin vs. Oralin in 10 type 1 diabetic subjects receiving baseline glargine insulin therapy

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Background and Aims: The aim of this pilot study was to determine the suitability of dose and formulation of Oralin for its use in a larger multicenter trial. We also compared the glucodynamics of both rapid insulins (Oralin and Humulin) in 10 Type-1 diabetic subjects receiving glargine insulin as their baseline therapy. Fructosamine, a parameter of protein glycation was determined as part of a panel of safety monitoring.
Materials and Methods: Subjects received their usual baseline glargine insulin therapy (2/3rd in the morning and 1/3rd in the evening). Depending on present glycemia, Humulin was dosed as a pre-meal s.c injection during days -3, -2 and -1. Five to eight puffs of Oralin were given pre- and postprandially on days +1 through +9. Adjustments of glycemia were done using standard snacks, additional s.c Humulin or Oralin puffs.
Results: Humulin and Oralin induced similar glucodynamic responses during the 12-day observation period.
Conclusion: Intensive monitoring and timely corrections resulted in an appropriate glycemic control as assessed by individual daily-glycemic curves and, especially, normal preprandial glycemia. Measurements of protein glycation displayed a tendency to lower values after the 12-day study period.

s.c HUMULIN (single dose)

	+6:55	+8:00	+9:55	+12:55	+14:00	+16:25	+20:25	+21:30	+22:10
MEAN	91.08	151.45	108.18	98.55	140.77	106.28	100.78	139.43	109.94
SD	27.80	55.78	40.87	27.56	33.36	33.26	17.68	22.61	47.30
ORALIN (split dose)									
	+6:55	+8:00	+9:55	+12:55	+14:00	+16:25	+20:25	+21:30	+22:10
MEAN	70.26	138.50	113.92	84.82	142.00	113.55	92.84	141.31	119.62
SD	13.77	24.51	33.50	14.29	23.07	26.92	16.46	25.39	26.07

OP 14

Links between microvascular and macrovascular complications

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Incident nephropathy in type 2 diabetes: a UKPDS Risk Equation

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Background and Aims: Many tools are available to estimate cardiovascular risk in clinical practice, including the UKPDS Risk Engine for patients with type 2 diabetes, but no tools have been developed for the risk of the microvascular complications of diabetes. We used UK Prospective Diabetes Study (UKPDS) data to develop a survival model for incident nephropathy, so that the UKPDS Risk Engine can estimate risk for a wider range of diabetic complications.
Materials and Methods: UKPDS patients had urine samples taken annually and plasma creatinine measured every three years. Of 5,102 there were 2,859 without baseline nephropathy who had the required covariate data available. Nephropathy was defined as: estimated glomerular filtration rate (eGFR) <60 ml/min (derived from plasma creatinine using the Cockroft-Gault formula); macroalbuminuria (urine albumin ≥300 mg/l on two successive measurements); or end stage renal disease. As the UKPDS recruited patients with newly diagnosed diabetes, this analysis randomly sampled them at varying time points to improve generalizability in populations with diabetes of different durations. Covariates considered for inclusion in the model were age, sex, Afro-Caribbean, Asian-Indian or White-Caucasian ethnicity, smoking status, HbA_{1c}, systolic blood pressure, ratio of total to HDL cholesterol and duration of diabetes. A multivariate Weibull survival model was fitted, allowing for interval censored data given that nephropathy status including eGFR could only be evaluated every three years.
Results: During mean 6 yrs follow-up there were 515 (18%) cases of incident of nephropathy (457 reduced eGFR, 58 macroalbuminuria). Significant univariate risk factors for nephropathy were age, female sex, systolic blood pressure and duration of diagnosed diabetes. These remained significant in a multivariate analysis (see table) with people of Afro-Caribbean (227) or Asian-Indian ethnicity (270) at significantly higher risk than White-Caucasians after adjustment for the other risk factors. Asian-Indian ethnicity was associated with lower mean (standard error) systolic blood pressure (127 (1.1) mm Hg) than White-Caucasian (137 (0.4) mm Hg). Afro-Caribbeans had a lower mean (standard error) age (50 (0.45) years) than White-Caucasians (52 (0.18) years). Smoking status, HbA_{1c}, and ratio of total to HDL cholesterol were not significantly associated with the risk of nephropathy.
Conclusion: Afro-Caribbean and Asian-Indian people with type 2 diabetes are at similar risk of incident nephropathy despite a more favourable risk factor profile, compared to White-Caucasians with type 2 diabetes. Correction for ethnicity, as well as age, sex and blood pressure is essential to assess the risk of nephropathy reliably in these groups. The UKPDS Risk Engine can now estimate risk of nephropathy in addition to macrovascular disease.

Risk Factors for Nephropathy

Risk Factor	Hazard Ratio	95% Confidence Interval
Age per year	1.12	1.10, 1.14
Female sex	1.91	1.56, 2.26
Afro-Caribbean	1.46	1.01, 1.92
Asian-Indian	1.64	1.03, 2.24
SBP per 10 mmHg	1.05	1.01, 1.10
Duration of diabetes per year	1.14	1.09, 1.18

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A SNP in the 3'UTR of the VEGF gene is associated with nephropathy in type 1 diabetic patients

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Background and aims: The gene encoding vascular endothelial growth factor (*VEGF*) has been suggested to play a role in the pathogenesis of diabetic microvascular complications like diabetic retinopathy and nephropathy (DN). VEGF induces vascular endothelial cell proliferation, migration and vasopermeability in many types of tissues including glomerular capillaries. The *VEGF* gene is located on chromosome 6p21, and its expression is regulated by a variety of factors, e. g. high glucose, hormones and cytokines. The aim of this study was to evaluate if single nucleotide polymorphisms (SNPs) of the *VEGF* gene are associated with type 1 diabetes (T1D), and its complication nephropathy.
Material and methods: A cross-sectional case-control pilot study was performed, focusing on "extreme" phenotypes; i.e. normoalbuminuric and macroalbuminuric patients. In this pilot study, 183 normo- with long duration (median=34 years) and 183 macroalbuminuric patients with short duration of T1D (median=23 years) from the ongoing FinnDiane Study were studied. To confirm the results of the pilot study additional genotyping including also microalbuminuric and end-stage renal disease (ESRD) patients was performed, 902 T1D patients (274 normo-, 271 micro-, 176 macroalbuminuric and 181 ESRD) were included. 364 non-diabetic healthy blood donors from different parts of Finland were genotyped and served as control subjects to the diabetic patients. Four evenly distributed SNPs in the *VEGF* gene were genotyped using TaqMan® technology. SPSS 11.5.1 was used for individual SNP analyses. Haplotypes were estimated by PHASE v.2.0.2 and analysed using Haplo-assoc.
Results: All studied SNPs were in Hardy-Weinberg equilibrium. In the pilot study, an association was seen between a SNP in the 3'UTR of the *VEGF* and DN. No association with DN was found for the other three SNPs studied. The frequency of the GG homozygots for the associated SNP was 21.3% in the normo- and 34.4% in the macroalbuminuric group (p=0.003). The haplotype analysis confirmed the results of the individual SNP analysis, the frequencies for the haplotypes containing the G-allele of the 3'UTR SNP differed significantly between the normo- and macroalbuminuric patients (p=0.001). However, in the replication study where also normoalbuminuric patients with shorter duration and macroalbuminuric patients with longer duration along with microalbuminuric and ESRD patients were included, no association was found between any of the *VEGF* SNPs and DN. No difference could be seen in the genotype distribution between the healthy controls and the diabetic patients in neither pilot or replication study, supporting the finding from the pilot study that the SNP is associated with DN and not T1D.
Conclusion: The observed association could indicate an involvement of the *VEGF* 3'UTR region in diabetic nephropathy in Finnish type 1 diabetic patients. However, the association was seen only when "extreme phenotypes" were considered.

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FPG and HbA_{1c} are independent risk factors for microvascular but not macrovascular complications in newly diagnosed type 2 diabetes

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Background and Aims: Individuals with HbA_{1c} values higher than expected for their fasting plasma glucose (FPG) are said to be at higher risk of complications than those with the same FPG but lower HbA_{1c}. We used data from the UK Prospective Diabetes Study (UKPDS) to determine whether HbA_{1c} and FPG are associated independently with incident microvascular and macrovascular complications in type 2 diabetes and used the haemoglobin glycation index (HGI) to summarise HbA_{1c} variation relative to FPG.
Materials and Methods: Of 5,102 UKPDS participants there were 3,538 with the required data. Microvascular events were defined as the first to occur of: retinopathy requiring photocoagulation; vitreous haemorrhage; or fatal or non-fatal renal failure. Macrovascular events were defined as the first to occur of: fatal or non-fatal myocardial infarction; fatal or non-fatal stroke; non-fatal ischaemic heart disease; or sudden death. Cox models were fitted for 313 microvascular and 766 macrovascular events observed during median 9.3 and 9.7 years follow-up, respectively. Potential confounders included in all models were post-dietary run-in values for age, sex, ethnicity, HDL and LDL cholesterol, triglycerides, systolic blood pressure and albuminuria (urine albumin ≥ 50 mg/l) and smoking status at time of diagnosis. FPG and HbA_{1c} were used in the first analysis as baseline values and in the second analysis as time-dependent variables (updated mean). HGI was calculated as actual HbA_{1c} minus the HbA_{1c} predicted from a linear regression equation fitted to baseline FPG.
Results: For microvascular complications, baseline FPG but not baseline HbA_{1c} was a predictor whereas updated mean HbA_{1c} and updated mean FPG were both independent predictors after adjustment for possible confounders (Table). For macrovascular complications, after adjustment for possible confounders, baseline FPG but not baseline HbA_{1c} was a predictor but only updated mean HbA_{1c} was a predictor. Median (1st, 3rd quartile) HGI was –0.069% (–0.75, 0.59) at baseline, 0.014% (–0.63, 0.72) at year 3, 0.27% (–0.38, 1.06) at year 6 and 0.47% (–0.18, 1.34) at year 9, showing that HbA_{1c} varies by more than 1% for individuals at the same FPG.
Conclusion: FPG and HbA_{1c} make independent contributions to predicting the risk of microvascular disease suggesting a role for the HGI, which measures disparity between HbA_{1c} and FPG as measures of glycaemia, in the management of type 2 diabetes. Updated mean HbA_{1c} is a strong independent predictor of macrovascular disease.
Relation between diabetic complications and FPG and HbA1c adjusted for other confounders at baseline

	Microvascular complications					
	Baseline FPG, HbA_{1c}			Updated mean FPG, HbA_{1c}		
Variables	Hazard ratio	95% CI	P-value	Hazard ratio	95% CI	P-value
FPG (per mmol/L)	1.17	(1.12, 1.22)	<.0001	1.16	(1.09, 1.24)	<.0001
HbA_{1c} (per %)	1.06	(0.98, 1.15)	0.17	1.30	(1.16, 1.44)	<.0001
	Macrovascular complications					
	Baseline FPG, HbA_{1c}			Updated mean FPG, HbA_{1c}		
Variables	Hazard ratio	95% CI	P-value	Hazard ratio	95% CI	P-value
FPG (per mmol/L)	1.06	(1.02, 1.10)	0.0035	1.04	(0.99, 1.09)	0.15
HbA_{1c} (per %)	0.97	(0.91, 1.03)	0.32	1.12	(1.03, 1.21)	0.0056

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No differences in 5 year mortality between diabetics with acute myocardial infarction and diabetics with chronic foot problems

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Background and Aims: The knowledge of higher mortality in diabetics compared to non-diabetics suffering from acute myocardial infarction has lead to more intense secondary prevention in diabetics with coronary heart disease in last decade. Despite the knowledge of high mortality risk in patients with diabetes and chronic foot ulcers, the interest for this disease and its complications has not been as high in the general health care system, as for coronary heart disease. The aim of this study was to compare five-year mortality between diabetics with acute myocardial infarction and diabetics attending the Multidisciplinary Diabetes Foot Team.
Materials and Methods: Helsingborg Hospital is the only hospital for 150 000 inhabitants in the southern part of Sweden. The Multidisciplinary Diabetes Foot Team at the Diabetes Ward treats all patients with diabetes and chronic foot problems. 5-year mortality was registered for all patients treated by the Multidisciplinary Diabetes Foot Team in 1997 and compared to 5-year mortality of all diabetics diagnosed with acute myocardial infarction at Department of Internal Medicine at Helsingborg Hospital in 1996 and 1997.
Results: 231 patients were treated by the Multidisciplinary Diabetes Foot Team (group 1) and 117 patients were diagnosed with acute myocardial infarction (group 2). Median age was 68 respectively 72 years. The five-year mortality rate was 43% in group 1 compared to 48% in group 2 (n.s).
Median ages in patients younger than 76 years were 68 respectively 63 years. The five-year mortality was 33% in group 1 and 35% in group 2 (n.s.).
Conclusion: The result of this study visualizes that chronic diabetes foot problems are as dangerous as acute myocardial infarction in diabetics regarding five-year mortality. Accordingly, these two groups of patients should be equally priorited by the health care system.

Mortality rate

	Group 1	Group 2
1 year mortality	10%	27%
2 year mortality	21%	39%
3 year mortality	29%	44%
4 year mortality	35%	45%
5 year mortality	43%	48%

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Need for echocardiographic screening of patients with diabetes mellitus and chronic foot ulcers

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Background and Aims: Diabetic patients with chronic foot ulcers display signs of neuropathy, peripheral artery disease or both linked to an increased cardiovascular morbidity and mortality. Using echocardiography our aim of the present study was to elucidate cardiac function in diabetic patients with chronic foot ulcers with or without known hypertension, previous myocardial infarction (MI) and/or heart failure.
Materials and Methods: Helsingborg Hospital is the only hospital for 150 000 inhabitants in the southern part of Sweden. All diabetics with chronic foot ulcers visit the Diabetic Foot Clinic. 50 consecutive patients with foot ulcers of at least three months duration underwent transthoracal echocardiography performed by the same cardiologist. Presence of left ventricular hypertrophy (LVH), reduced ejection fraction (EF) and diastolic dysfunction were registered. Patients were divided into groups depending on known presence of hypertension, previous MI or heart failure.
Results: Median age 70 years.

Patient Group	Normal	Reduced EF	LVH	Diastolic Dysfunc.	2 or more
Known hypertension– 34%	23%	41%	41%	53%	47%
Known heart failure – 22%	19%	64%	55%	73%	73%
No previous MI, heart failure or hypertension – 38%	37%	21%	47%	53%	53%

Conclusion: Using echocardiography, signs of cardiac dysfunction was visualised in all patient groups. Accordingly, to optimise diagnosis and thereby treatment, the need for echocardiography as a diagnostic tool may be recommended in all diabetic patients with chronic foot ulcers.

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Impaired cognition in type 2 diabetes: MRI correlates and vascular risk factors

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Background and Aimes: Type 2 diabetes (DM2) is associated with cognitive deficits and dementia, particularly in the elderly. These impairments are frequently attributed to ischaemic cerebrovascular disease. However, conclusive data on the structural correlates of these cognitive impairments, and on the association with vascular risk factors were still lacking. We aimed to collect these data.
Patients and Methods: Patients were recruited through their general practitioner. For the present study 122 DM2 patients (average age 66) and 59 non-DM controls (matched for age, sex and education) were included. A neurological and neuropsychological examination and an MRI-scan of the brain were performed, and indices of vascular function recorded, including vascular history and smoking habbits, blood pressure, and diabetic microvascular changes. Also, diabetes related risk factors were analysed.
Results: Hypertension (73 versus 32%) was more common in the DM2 group. Also, macrovascular disease was more common in patients with DM2: MI or CABG (19% against 3%), stroke (19% against 7%), claudicatio intermittens or arterial surgery to the legs/abdomen (15% against 0%). Moderate cognitive dysfunction was observed in DM2 patients, particularly affecting executive functioning, information processing speed and memory (effect sizes 0.2 to 0.4). On MRI atrophy (cortical $p<0.001$, subcortical $p<0.001$) white matter lesions (deep: $p<0.01$ periventricular: not significant), and (silent) cerebral infarcts (19 versus 7%) were more common in patients than in controls. Within the DM2 group cognitive function was inversely related with white matter lesions, subcortical atrophy and the presence of infarcts (linear regression analysis, corrected for age, education, sex), and to a lesser extent with cortical atrophy.
Hypertension was related with periventricular white matter lesions, but not with cognitive impairments. Macrovascular disease was associated with deep white matter lesions and impairments in information processing speed. No other vascular factors were related with cerebral deficits. Diabetes duration and HbA1c were not related with cerebral deficits.
Conclusions: Cognitive impairment in DM2 is related to subcortical (vascular) MR abnormalities and to a lesser extent to cortical atrophy. This relation appears to be partly dependent of macrovascular disease and to a lesser extent hypertension, and to be largely independent of diabetes related disease variables.
Support: Dutch Diabetes Research Foundation, grant: 2001.00.023

OP 15
Adiponectin in humans

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Clinical implications of serum high-molecular-weight (HMW) adiponectin concentrations in patients with type 2 diabetes: a novel assay for detecting high-molecular-weight adiponectin

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Background and Aims: Adiponectin (Acrp30), an adipocyte-derived protein, exists in at least two oligometric isoforms in serum as a hexamer (low-molecular weight [LMW] form) and a higher form of between12–18 subunits (HMW form). We measured serum HMW and total adiponectin concentration in patients with type 2 diabetes, using a novel ELISA system, and investigated the relationship between HMW or LMW adiponectin in sera and blood inflammatory markers including high-sensitivity C reactive protein (hs-CRP) and IL-6.
Research Design and Methods: We studied 105 type 2 diabetic patients (45 patients with normoalbuminuria, 32 with microalbuminuria, and 28 with macroalbuminuria). Serum total adiponectin was measured as previously described. We established a novel ELISA method for detecting only HMW adiponectin in sera and confirmed the validity of this assay. We calculated serum LMW adiponectin: total adiponectin- HMW adiponectin.
Results: Linear regression analysis showed that serum HMW adiponectin, but not LMW adiponectin, correlated positively urinary albumin excretion, plasma fibrinogen, and IL-6, while serum LWM adiponectin, but not HMW adiponectin, correlated negatively with BMI, triglyceride, free fatty acid, PAI-1, and hs-CRP. Serum total and HMW adiponectin, but not LMW adiponectin, were significantly higher in diabetic patients with overt nephropathy than in those with normo- and microalbuminuria. Plasma fibrinogen and IL-6, but not hs-CRP, were significantly higher in diabetic patients with overt nephropathy than in those with normo- and microalbuminuria, suggesting the dissociation in concentrations of blood inflammatory markers in overt diabetic nephropathy.
Conclusions: Serum HMW adiponectin was influenced by renal function, whereas serum LMW adiponectin was related closely with components of metabolic syndrome, suggesting that different isoforms of adiponectin in sera may have different activities on metabolic syndrome.

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Reduced plasma adiponectin concentrations may contribute to impaired insulin activation of glycogen synthase in skeletal muscle of patients with type 2 diabetes

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Background and Aims: Circulating levels of adiponectin are negatively associated with several measures of insulin resistance, and the concentration is reduced in humans with insulin resistance and type 2 diabetes mellitus (T2DM). However, the physiological and molecular mechanisms by which adiponectin improve insulin sensitivity remain to be established.
Materials and Methods: We examined the relationship between plasma adiponectin and parameters of whole-body glucose and lipid metabolism, as well as enzyme activity of muscle glycogen synthase in 10 lean and 20 obese healthy subjects, and 20 obese patients with T2DM before (baseline) and after a 4-h euglycemic-hyperinsulinemic clamp. In addition, the relationship between plasma adiponectin and alpha-2 AMPK activity in muscle was studied in subpopulations of 10 obese non-diabetic and 10 obese type 2 diabetic male subjects
Results: Plasma adiponectin was reduced in patients with T2DM (5.7 ± 0.5 vs. 8.7 ± 1.1 vs. 10.6 ± 1.6 mg/l) compared to obese ($p=0.02$) and lean ($p<0.01$) subjects, respectively. Insulin infusion reduced plasma adiponectin in lean (10.6 ± 1.6 to 9.8 ± 1.5 mg/l, $p<0.001$) and obese subjects (8.7 ± 1.1 to 8.2 ± 1.0 mg/l, $p=0.003$), whereas adiponectin remained unchanged in patients with T2DM. In the total population baseline plasma adiponectin was positively associated with HDL cholesterol ($r=0.59$, $p<0.001$), insulin-stimulated glucose disposal ($r=0.40$, $p<0.01$), glucose oxidation ($r=0.51$, $p<0.001$), and glucose storage ($r=0.27$, $p=0.05$) and negatively associated with fasting plasma glucose ($r=-0.39$, $p<0.01$), serum insulin ($r=-0.42$, $p<0.01$), plasma triglyceride ($r=-0.32$, $p=0.02$), insulin-suppressed endogenous glucose production ($r=-0.56$, $p<0.001$), and lipid oxidation ($r-0.55$, $p<0.001$). Interestingly, plasma adiponectin correlated positively with insulin-stimulated fractional velocity (FV) of muscle glycogen synthase (GS) ($r=0.36$, $p=0.01$), and the incremental increase from baseline in GS FV ($r=0.39$, $p<0.01$). In a subpopulation of 10 obese non-diabetic male subjects, in which correlation between plasma adiponectin and the incremental increase in GS FV during clamp was conserved ($r=0.70$, $p=0.03$), we also found a significant correlation between adiponectin levels and alpha-2 AMPK activity ($r=0.79$, $p<0.01$).
Conclusion: This investigation confirms earlier studies showing an association between circulating adiponectin and several measures of insulin resistance, as well as a negative effect of insulin infusion on plasma adiponectin. In particular, this study provides support for an effect of adiponectin on AMPK activity in human skeletal muscle, and it demonstrates for the first time a potential role of adiponectin in insulin action on GS activity. Thus, in obese patients with T2DM reduced levels of adiponectin may contribute to skeletal muscle insulin resistance via an impaired insulin activation of glycogen synthase.

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Hypoadiponectinemia is closely related to impaired nitric oxide synthase (NOS) activity in skeletal muscle of type 2 diabetic subjects

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Background and Aims: Type 2 diabetes (T2DM) is an insulin-resistant state characterized by decreased plasma adiponectin concentration and accelerated atherosclerosis. *In vitro* and *in vivo* studies in rodents have suggested that adiponectin plays an important role in nitric oxide (NO) generation. We determined nitric oxide synthase (NOS) activity in skeletal muscle of type 2 diabetic and healthy non-diabetic control subjects under basal and insulin-stimulated conditions. The results were related to whole body (muscle) insulin sensitivity and plasma adiponectin concentration.
Materials and Methods: Seven type 2 diabetics (age = 51 ± 4 y, BMI = 31.3. ± 1.0 kg/m^2, HbA_{1c} = 6.8 ± 0.9%) and 8 healthy gender/ethnic-matched controls (age = 45 ± 4 y, BMI = 29.5 ± 0.9 kg/m^2, HbA_{1c} = 4.9 ± 0.2%) received an 80 mU/m^2·min euglycemic insulin clamp to measure the rate of insulin-stimulated whole body glucose disposal (Rd); vastus lateralis muscle biopsies were performed before and after 4 hr of insulin. Basal plasma adiponectin concentration was determined by RIA. Muscle NOS activity was measured using a labeled L-arginine to citrulline conversion assay, and NOS protein content was assessed by immunoblot analysis.
Results: Rd (5.2 ± 0.4 vs. 9.0 ± 0.9 mg/kg^{-min}, $P < 0.01$) and basal NOS activity was reduced in T2DM vs. controls (107 ± 45 vs. 459 ± 100 pmol/min · mg protein, $P<0.05$). In response to hyperinsulinemia, NOS activity increased almost 2-fold in controls after 4 hours (757 ± 244 pmol/min · mg protein, $p<0.05$ vs. basal) but failed to increase in T2DM (104 ± 38 pmol/min·mg protein, $p<0.01$ vs. T2DM). Basal NOS protein content in muscle was similar in controls and T2DM and did not change following insulin. Plasma adiponectin conc was decreased in T2DM (4.5 ± 0.8 vs. 7 ± 1.0 mg/kg^{-min}, $P < 0.02$) and correlated positively with insulin-stimulated NOS activity ($r = 0.49$, $P < 0.05$) and with Rd ($r = 0.50$, $P < 0.05$). In control and T2DM collectively, Rd correlated with insulin-stimulated NOS activity ($r = 0.48$, $P < 0.05$).
Conclusion: Basal and insulin-stimulated muscle NOS activity are reduced in well-controlled T2DM. Decreased plasma adiponectin conc correlates positively with the impaired insulin-stimulated NOS activity and with the severity of insulin resistance. Since impaired nitric oxide generation represents an early defect in the development of atherosclerosis, our results may provide a link between reduced adiponectin levels and accelerated atherosclerosis in individuals with T2DM.

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Adiponectin expression in subcutaneous and visceral adipose tissue in severely obese subjects with or without diabetes

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Background and Aims: Adiponectin, an adipocyte-specific protein associated with insulin sensitivity, is thought to play a role in mediating the metabolic effects of obesity. Whether adiponectin (APM1) expression in subcutaneous (APM1 SC) and visceral (APM1 Vis) adipose tissue differs between lean subjects and severely obese subjects with or without type 2 diabetes has not been determined.
Materials and Methods: Total RNA was extracted from human tissue specimens (SC and Vis), obtained during abdominal surgery, from 6 lean non-diabetic subjects (C) (47 ± 12 years, BMI 24.1 ± 1.8 kg/m^2), 10 obese diabetic subjects (DM2-Ob) (49 ± 9 years, BMI 44.9 ± 8.0 kg/m^2) and 10 obese subjects with normal glucose tolerance (Ob) (37 ± 12 years, BMI 46.2 ± 5.5 kg/m^2). cDNA was synthesised and relative quantification of APM1 mRNA (target gene) was performed by Real-Time PCR. Results were expressed as the target/reference (APM1/Hypoxantine phosphorybosyltransferase) ratio for each sample.
Results: As expected, fasting plasma glucose concentrations were higher in diabetic subjects compared to non-diabetic obese and control individuals (144 ± 35 vs 98 ± 26 and 87 ± 11 mg/dl, respectively, p<0.0007 for DM2-Ob vs Ob or C, p=ns for Ob vs C). In subcutaneous adipose tissue, APM1 expression was significantly higher in lean control subjects than in diabetic or non-diabetic obese individuals, whereas no difference was found between DM2-Ob and Ob subjects (1.45 ± 0.8 vs 0.68 ± 0.72 and 0.86 ± 0.68 respectively, p=0.06 for C vs Ob and p=0.02 for C vs DM2-Ob). Similarly, APM1 was significantly more expressed in visceral adipose tissue from lean controls compared to diabetic and non-diabetic obese subjects and no difference was detected between DM2-Ob and Ob groups (1.88 ± 1.3 vs 0.66 ± 0.53 and 0.78 ± 0.66 respectively, p= 0.02 for C vs Ob and p=0.01 for C vs DM2-Ob). Furthermore, a positive correlation was found between APM1 expression in subcutaneous or visceral fat and BMI (APM1 SC vs BMI: r=0.42, p=0.06 and APM1 Vis vs BMI: r=0.46, p=0.02). No difference was found in the expression of APM1 in subcutaneous and visceral fat between women and men with severe obesity regardless of diabetic status (APM1 SC: 0.87 ± 0.54 vs 0.64 ± 0.87, p=ns; APM1 Vis: 0.75 ± 0.65 vs 0.66 ± 0.48, p=ns).
Conclusion: Adiponectin gene expression is similar in the two different fat depots (visceral and subcutaneous) independently of obesity and diabetic status. In severe obesity, adiponectin expression is lower than in lean individuals both in subcutaneous and visceral fat; moreover, no difference in the APM1 expression was found between obese subjects with or without diabetes in either adipose depot.

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Plasma concentrations and expressions of selected adipokines in subcutaneous adipose tissue in healthy subjects during 24-hour hypertriglyceridaemia

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Background and Aims: Several adipokines and growth factors are implicated in the pathophysiology of insulin resistance. The aims of the study are: a) to measure plasma concentrations of TNFα, leptin, resistin and adiponectin and their real-time expression in abdominal subcutaneous adipose tissue and b) to test their responses to prolonged hypertriglyceridaemia
Materials and Methods: 8 lean healthy subjects underwent 24-hour-lasting infusion of lipid emulsion (Intralipid 20%; 3 g of fat · kg^{-1} · d^{-1}). Plasma concentrations of TNFα, leptin, resistin and adiponectin were measured before (0 min), at 30 min, 240 min and 24 hours of infusion. Needle biopsy of abdominal subcutaneous adipose tissue was performed before, at 240 min and 24 hours of infusion to asses the expression of selected cytokines by real-time PCR (expression of adipokine mRNA relative to cyclophilin mRNA).
Results: TNFα plasma concentration didn't change during first 4 hours of hypertriglyceridaemia, but a significant increase after 24 hours was detected (0; 30; 240 min; 24 h: 1.20 ± 0.22 vs. 1.14 ± 0.28 vs. 1.31 ± 0.25 vs. 2.53 ± 0.73 pg · ml^{-1}; p<0.01 for 0; 30; 240 min vs. 24 h). Plasma concentration of resistin significantly increased at 30 min of infusion and remained at the same level throughout 24 hours (0; 30; 240 min; 24 h: 5.93 ± 5.64 vs. 7.54 ± 6.77 vs. 7.25 ± 6.17 vs. 8.93 ± 5.67 ng · ml^{-1}; p<0.01 for 0 min vs. 30; 240 min; 24 h). Significant positive correlation has been found between the increase in resistin concentration during lipid infusion and basal LDL-cholesterol levels in plasma (r + 0.846; p<0.01). Although the expression of resistin in the subcutaneous adipose tissue tended to increase, the change was not significant (0; 240 min; 24 h: 9.61 ± 8.42 vs. 12.25 ± 13.36 vs. 17.25 ± 13.67). Expression of the remaining adipokines (TNFα, leptin and adiponectin) seemed rather unaffected. Plasma concentrations of leptin and adiponectin didn't show any significant changes.
Conclusion: In healthy subjects, 24-hour hypertriglyceridaemia results in an increase in resistin and TNFα plasma concentrations. This can not be fully explained by changes in expression in abdominal subcutaneous adipose tissue. We conclude that the main changes in secretion of selected adipokines take place at a different fat depot.
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Adiponectin is a marker of insulin resistance and increased risk of atherogenesis in obese children

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Background and Aims: Adiponectin (AD) is an adipocytokine inversely related to adiposity with proved insulin-sensitizing, anti-inflammatory, and atheroprotective effects in adults. The role of AD in the development of insulin resistance and inflammation in children is still unclear.
Materials and Methods: In 162 obese children, 41% males, 8–18 yr, SDS BMI 3.7 ± 0.51, we measured serum levels of adiponectin, interleukin 18 (IL-18), PAI-1, CRP, fibrinogen and lipids. All children underwent an OGTT. Insulin resistance was measured by homeostasis model assessment (HOMA-IR) and insulin secretion by insulinogenic index. The metabolic syndrome (MS) was defined using WHO-derived child based criteria.
Results: Serum AD was lower in boys than in girls (8.4 ± 0.54 vs 11.0 ± 0.61 μg/ml, p<0.005) and similar in the different pubertal stages as well as in subjects with and without family history of diabetes. In the univariate analysis, AD was negatively correlated with SDS BMI, HOMA-IR, PAI-1, triglycerides (r–0.315, r–0.274, r –0.352, r–0.254, p<0.001 for all), fibrinogen, 2 h glucose and insulinogenic index (r –0.213, r –0.216, r–0.183, p<0.05 for all) and positively with HDL cholesterol (r 0.342, p<0.001). In the multivariate analysis with AD as dependent variable, only HOMA-IR was independently related to AD (beta coefficient –0.471, p<0.005). When the whole cohort was divided into tertiles of SDS BMI and further stratified on the basis of the tertiles of HOMA-IR, obese children in the third tertile of HOMA-IR were characterized by significantly lower levels of AD than those in the first tertile (7.4 ± 1.50 vs 12.5 ± 1.57, p<0.05). Twenty five percent of obese children had the MS. After adjustment for SDS BMI and sex, obese children with MS compared to those without the syndrome had lower levels of AD (7.5 ± 0.98 vs 10.8 ± 0.46 μg/ml, p<0.005) and higher levels of HOMA-IR, insulinogenic index (4.4 ± 0.28 vs 2.3 ± 0.17 and 2280 ± 139 vs 1778 ± 82, p<0.0001 for both) and IL-18, PAI-1 (388.4 ± 39.1 vs 247.6 ± 14.4 pg/ml, p<0.01; 199.5 ± 1.14 vs 125.9 ± 1.07 ng/ml, p<0.05). Serum levels of AD decreased while HOMA-IR, insulinogenic index, PAI-1 and IL-18 significantly increased with the number of the components of MS.
Conclusion: These results suggest that in obese children a) the degree of obesity is not a good marker of insulin resistance and measuring AD might help to identify those who have insulin resistance, b) lower levels of AD are associated with MS and with several factors involved in development of atherogenesis, independently of the degree of obesity.

OP 16

Signal transduction of beta cell death

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Suppressor of cytokine signalling (SOCS)-3 inhibits the beta-cytotoxic IL-1 signalling by targeting the TRAF6/TAK1 complex

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Background and Aims: The proinflammatory cytokine IL-1β is an important mediator of beta-cell destruction in Type 1 Diabetes Mellitus and induces beta-cell death in an NFκB and MAPK dependent manner. While a great deal is known about the mechanism of activation of NFκB and MAPKs by IL-1, much less is known about the down-regulation of this pathway. The protein Suppressor Of Cytokine Signalling-3 (SOCS-3) belongs to a family of negative feedback inhibitors of cytokine signalling. We have previously shown that SOCS-3 inhibits IL-1 induced apoptosis in beta-cells. The aim of this study was to investigate the mechanisms whereby SOCS-3 exerts its inhibitory effect on IL-1 induced signalling.
Materials and Methods: A beta-cell line, with inducible expression of SOCS-3 and HEK 293 cells transfected with SOCS-3 were used. Phosphorylation of the MAPKs and degradation of IκB in the presence and absence of IL-1 and SOCS-3 was measured by Western Blotting, as was the ubiquitination of TRAF6. Furthermore, NFκB dependent transcription induced by expression of several IL-1 signalling molecules in the presence or absence of SOCS-3 was analysed using an iNOS promoter-luciferase reporter assay. Lysates from transfected HEK293 cells were co-immunoprecipitated to analyse the interaction between TAK1 and TRAF6, and TAK1 and SOCS-3, and the activity of TAK1 in the presence or absence of SOCS-3 was analysed in an *in vitro* kinase assay.
Results: SOCS-3 inhibited both the NFκB and MAPK branch of the IL-1 signalling pathway. Furthermore, SOCS-3 inhibited NFκB dependent transcription induced by over-expression of the upstream IL-1 signalling molecules MyD88, IRAK1, TRAF6 and TAK1, but not when the down-stream signalling molecule MEKK is used, indicating that the target for SOCS-3 is the TRAF6/TAK1 signalling complex. SOCS-3 inhibited the association between TRAF6 and TAK1 and was co-immunoprecipitated with TAK1. Furthermore, SOCS-3 inhibited the IL-1 induced catalytic activity of TAK1. As ubiquitination of TRAF6 is required for activation of TAK1 we analysed the role of SOCS-3 on TRAF6 ubiquitination and found that SOCS-3 inhibited ubiquitin modification of TRAF6.
Conclusion: The results indicate that SOCS-3 inhibits IL-1 signal transduction by inhibiting ubiquitination of TRAF6, thus preventing association and activation of TAK1 and thereby further signalling. By elucidating how SOCS-3 protects against the inflammatory effects of IL-1, SOCS-3 could represent a therapeutic target that may alter the course of inflammatory diseases, including T1DM.
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Influence of SOCS-3 on cytokine effects in pancreatic beta cells

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Background and Aims: Preservation of the pancreatic beta cell mass is an important issue in relation to both Type 1 and Type 2 diabetes. The beta cell population is a dynamic structure changing with the body's varying demand for insulin and it is regulated by three processes namely, 1) neoformation of beta cells from pancreatic stem cells, 2) replication of preexisting beta cells, and 3) beta cell death. Different cytokines can influence these processes; for example growth hormone (GH) can stimulate beta cell proliferation whereas the pro-inflammatory cytokines interleukin 1 beta (IL-1) and interferon gamma (IFN-g) can induce beta cell apoptosis. Suppressors of cytokine signaling (SOCS) proteins are a family of proteins known to regulate cytokine signaling. The aim of the present work has been to address the potential role of SOCS-3 on cytokine signaling in pancreatic beta cells.
Materials and Methods: Transgenic mice with beta cell-specific SOCS-3 expression (RIP-SOCS-3 mice) and cultured primary beta cells transduced with a SOCS-3 expressing adenoviral (AdV) construct were used in order to investigate whether SOCS-3 could influence GH, IL-1 and IFN-g effects in beta cells.
Results: By morphometry, the relative beta cell volume of RIP-SOCS-3 transgenic mice was measured. Interestingly, the beta cell volume of transgenic female mice was reduced by more than 30% when compared to that of wild-type female littermates. In agreement with this, the pancreatic insulin content was likewise found to be reduced in transgenic female mice compared to wild-type littermates. GH is known to induce beta cell proliferation through STAT-5 activation. When transgenic islets cultured *in vitro* were stimulated with GH, a reduction in GH-induced STAT-5 tyrosine phosphorylation was seen when compared to wild-type islets. Moreover, when monolayer cultures of primary beta cells were transduced with SOCS-3 expressing AdV, GH-induced proliferation was diminished in these cells when compared to non- or luciferase AdV transduced cultures. These results indicate that the reduced beta cell volume seen in transgenic female mice could be explained by SOCS-3 mediated inhibition of GH-induced beta cell proliferation. Type 1 diabetes is caused by an autoimmune destruction of the pancreatic beta cells involving the pro-inflammatory cytokines IL-1 and IFN-g. IL-1 is known to induce NFkB dependent iNOS expression in beta cells, resulting in impaired insulin release, NO-production and beta cell death - an effect potentiated by IFN-g. However, islets isolates from RIP-SOCS-3 transgenic mice showed significant resistance to these toxic effects of IL-1+IFN-g when compared to wild type islets. Moreover, by means of primary beta cells grown as a monolayer and exposed to SOCS-3 expressing AdV it was shown that SOCS-3 was able to inhibit IL-1 and IL-1+IFN-g induced NO-production and apoptosis. These results demonstrate that SOCS-3 can protect beta cells from the cytotoxic effect of inflammatory cytokines.
Conclusion: The present study demonstrates a reduction of the beta cell mass in female RIP-SOCS-3 mice and that SOCS-3 inhibits GH-induced STAT-5 activation and proliferation of primary beta cells. Furthermore, SOCS-3 was able to inhibit IL-1 and IFN-g induced apoptosis of primary beta cells. Accordingly, SOCS-3 might be an important target in attempts to preserve the pancreatic beta cell mass.

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Toll-like receptor 3 and STAT-1 contribute for dsRNA+IFN-γ-induced apoptosis in pancreatic beta cells

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Background and Aims: Viral infections and local production of cytokines contribute to the pathogenesis of Type 1 diabetes. We have shown that the viral replicative intermediate dsRNA (tested in the form of polyinosinic-polycytidylic acid; PIC) triggers beta cell apoptosis when used in combination with interferon-γ (IFN-γ). The Toll-like receptor 3 (TLR3) is the cellular dsRNA receptor, and stimulation of TLR3 by dsRNA in other cell types induces NF-κB activation and production of type I IFNs. We observed by microarray analysis that dsRNA induces expression of several genes downstream of the TLR3 pathway in beta cells. We presently examined the role of TLR3 in primary beta cells and in INS-1E cells, a cell line resistant to the deleterious effects of dsRNA.
Materials and Methods: FACS-purified beta cells isolated from rat or mice (wild type or STAT-$1^{-/-}$) and INS-1E cells were cultured for 1 to 6 days in the presence of PIC (100 μg/ml), and/or IFN-α (500 U/ml), IFN-β (500 U/ml) or IFN-γ (500 U/ml). In some experiments, an antibody raised against IFN-β (20 μg/ml) was added. The percentage of viable, apoptotic and necrotic cells was determined by the nuclear dyes HO 342 and propidium iodide. After time-courses experiments (1, 2, 6 and 24 hours), expression of TLR3, IFN-α and IFN-β was determined by RT-PCR. Activation of NF-κB, STAT and of the IFN-β promoter was studied by luciferase reporter assay in the presence or absence of a dominant negative form of TRIF, the TLR3 specific adaptor molecule.
Results: Exposure of beta cells or INS-1E cells to PIC alone did not affect cell viability; together with IFN-γ, however, PIC significantly increased apoptosis (40% vs 9% in control cells; $p<0.005$). Beta cell exposure to a combination of PIC + IFN-α or -β induced a similar degree of apoptosis as PIC+IFN-γ (32 and 34% respectively; $p<0.05$ vs ctrl). INS-1E cells were resistant to the combined action of PIC+IFNs. In primary beta cells, a 6 hours exposure to PIC (100 μg/ml) increased TLR3 mRNA expression by 4-fold ($p<0.01$ *vs* ctrl). dsRNA also induced a significant and sustained

increase in IFN-β mRNA expression, starting already at 1 hour ($p<0.05$ vs ctrl) and addition of an antibody against IFN-β partially prevented PIC+IFN-γ-induced beta cell death ($p<0.05$ vs ctrl). Exposure to dsRNA activated NF-κB and STATs reporters (6 and 3 fold increase respectively; $p<0.05$ vs ctrl) and the IFN-β promoter (7 fold increase; $p<0.01$ vs ctrl). TRIF was required for NF-κB and IFN-β activation, and beta cells obtained from STAT-$1^{-/-}$ mice were protected against PIC+IFN-γ-induced apoptosis. On the other hand, dsRNA did not activate expression of TLR3 and type I IFNs mRNAs, or induced NFκB activation in INS-1E cells.
Conclusions: 1. Exposure of beta cells to dsRNA in combination with IFN-α, -β or -γ induces apoptosis; 2. In primary beta cells, dsRNA induces TLR3 and IFN-β mRNA expression and activates the transcription factors NF-κB and STATs, and the IFN-β promoter; 3. Disruption of the STAT-1 or the IFN-β signalling pathways protects beta cells against dsRNA+IFN-γ-induced apoptosis; 4. dsRNA+IFNs does not induce apoptosis in INS-1E cells which do not express TLR3. 5. These data suggest that dsRNA + IFN-γ triggers beta cell apoptosis by two complementary pathways, namely TLR3-TRIF-NF-κB and STAT-1.
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Nitric oxide causes activation of JNK and suppression of Akt in insulin-secreting cells
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Background and Aims: Pro-inflammatory cytokines including interleukin-1 (IL-1) and interferon are considered to be key immune mediators of β-cell death in type 1 diabetes. *In vitro* exposure of β-cells to cytokines stimulates the expression of inducible nitric oxide synthase (iNOS) leading to production of NO and eventually cell death. However, the mechanisms underlying NO-mediated β-cell death are unclear. Here, we investigated whether NO affects the pro-apoptotic c-jun N-terminal kinase (JNK) and the survival Akt signalling pathways.
Materials and Methods: Rat insulin-secreting INS-1 cells were exposed to various concentrations of the NO donor *S*-nitroso-*N*-acetyl-D,L-penicillamine (SNAP) or to IL-1. Cell death was determined by ELISA detection of histone-DNA complexes in the cytoplasm. The activation state of JNK and Akt was determined by Western blot analysis of Thr183/Tyr185- and Ser473-phosphorylated JNK and Akt, respectively.
Results: SNAP caused a dose- and time-dependent reduction in the cellular content of phosphorylated Akt in INS-1 cells. In contrast, SNAP induced phosphorylation of JNK. In isolated rat islets, inhibition of endogenous NO production reduced IL-1-induced sustained JNK activity. Similarly, IL-1-induced sustained JNK signalling was decreased in mouse islets from iNOS knock out animals as compared to wild type islets, suggesting that NO is important for sustained IL-1-induced JNK signalling. Apoptosis in INS-1 cells induced by NO was significantly reduced upon co-incubation with insulin-like growth factor (IGF)-1, an activator of Akt signalling. The protective effect against NO-induced apoptosis by IGF-1 was reversed by the PI3K inhibitor LY294002, demonstrating the importance of the PI3K - Akt signalling pathway in the anti-apoptotic effect of IGF-1. NO-induced activation of JNK was not affected by IGF-1, suggesting that downstream survival effects of Akt are not associated with suppression of pro-apoptotic JNK signalling.
Conclusion: Our findings suggest that NO contributes to cytokine-induced β-cell apoptosis via activation of JNK and suppression of Akt signalling.
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Uncoupling protein 2 overexpression prevents cytokine-induced reactive oxygen species production and apoptosis in pancreatic beta cells
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Background and Aims: Excessive apoptosis is the main process of β-cell death leading to type I diabetes and recent studies suggest that this may also be the case for type II diabetes. Several studies demonstrate that the mitochondria and its generation of reactive oxygen species (ROS) plays a central role in the apoptotic process induced by cytokines, and probably also by long term exposure to fatty acids and/or high glucose. Any factor able to decrease ROS production could therefore prevent or attenuate cell death induced by cytokines, fatty acids or high glucose. ROS production is highly dependant on the mitochondrial membrane potential and recent data demonstrate that ROS are also the main activators of uncoupling protein 2 (UCP2), the uncoupling protein expressed by the pancreatic β-cells. Activation of UCP2 leads to a decrease of the mitochondrial membrane potential and this, in turn will decrease ROS production.
The aim of this study is to investigate if UCP2 could be a good candidate as a protector against agents acting on cell viability by increasing ROS production like cytokines, in particular IL-1β.
Materials and Methods: INS 1 R9 cell expressing a Tet-On system were transfected with a plasmid containing UCP2. This allows for graded expression of UCP2 (2 to 12 fold) with increasing concentrations of doxycycline. After the induction of UCP2, the cells were cultured with IL1-β +/– IFN-γ and cell viability (MTT assay), active caspase 3 (Western blotting), ROS (CM-H2DCFDA) and NO production (Griess reagent) measured after 24 or 48 h.
Results: Exposure of control cells to IL-1β (250 pg/ml) results in a decreased cell viability after 24 h. UCP2 overexpression significantly ($p < 0.02$) decreases cell death induced by the cytokine. This effect is proportional to the expression of UCP2. Measurement of active casapse 3 confirmed that cells with high levels of UCP2 were protected from cytokines-induced apoptosis Exposure to IL-1β induces iNOS and increases the production of NO. NO production in response to IL-1β 250 pg/ml was significantly inhibited by the induction of UCP2. IL1 β -induced ROS production was decreased by 50% in cells overexpressing UCP2. All these parameters were also measured in INS-1 cells overexpressing either UCP1 or UCP3 and similar results obtained.
Conclusion: These data suggest that an increase in UCP2 could prevent cell death in response to cytokines by decreasing the production of ROS and of nitric oxide, and by reducing the activation of caspase 3.
The fact that similar results are obtained with the 2 other isoforms of uncoupling protein indicate that decrease in ROS production may be a common property of the 3 uncoupling protein.
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Selective inhibition of nuclear factor-κB activity in β-cells prevents cytokine induced apoptosis and protects mice from multiple low dose streptozocin induced diabetes
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Background and Aims: Type 1 diabetes is characterized by infiltration of inflammatory cells into the pancreatic islets followed by selective destruction of β-cells. Cumulative evidence suggests that the transcription factor NF-κB is, at least in part, responsible for triggering this process. Islet-infiltrating leukocytes secrete cytokines such as IL-1β and IFN-γ. These cytokines activate NF-κB leading to formation of radicals and expression of pro-apoptotic genes suggested to be involved in β-cell destruction. NF-κB is retained in the cytoplasm of resting cells by its inhibitor-IκB. Cell stimulation results in rapid phosphorylation, ubiquitination, and degradation of IκB, freeing NF-κB to translocate to the nucleus where it regulates transcription of target genes. Our aim is to evaluate the role of NF-κB in promoting β-cell resistance to damaging effects both under *in vivo* and *in vitro* conditions.
Materials and Methods: We generated a transgenic mouse model expressing a degradation-resistant IκBα (ΔNIκBα) specifically in β-cells in an inducible and reversible manner (using the tet-on system). Its expression is externally controlled by the administration of the tetracycline analog doxycycline (Dox) in the drinking water. Isolated islets from Dox-treated or untreated mice where exposed to a combination of pro-apoptotic cytokines IL-1β (50 U/ml) and IFN-γ (1000 U/ml) for 2–3 days and the degree of β-cell apoptosis was assessed using the TUNEL assay. The protective effect of NF-κB inhibition was further examined *in-vivo* using MLDS (50 mg/kg bw on 5 consecutive days) in Dox-treated or untreated mice.
Results: Dox-treated transgenic mice were normoglycemic even after prolonged exposure to doxycycline. IPGTT was normal after 3 days of doxycycline administration. Blocking NF-κB activation significantly protected islets from cytokine-induced nitrite secretion and apoptosis. Untreated islets exposed to cytokines for 2 days released 6.1 ± 0.2 μM nitrite in the medium, while Dox-treated islets released only 4 ± 0.28 μM nitrite ($n = 8–12$; $p<0.05$). Basal nitrite release from control (i. e. non-cytokine-treated)

with or without Dox treatment was in the range of 3 ± 0.3 μM. Under the same conditions, Dox-treated islet β-cells were significantly more resistant to cytokine-induced apoptosis than untreated β-cells (8.1% *vs* 24%; n = 4; p<0.05). We further evaluated the protective capabilities of NF-κB inhibition *in-vivo* in a MLDS- induced diabetes model. Dox-treated mice were considerably more resistant to multiple low dose streptozocin induced diabetes (1/17 diabetic in the Dox-treated group *vs* 10/15 untreated; P<0.05). No protective effect of NF-κB inhibition was observed after a single high dose (150 mg/kg bw) streptozocin injection.
Conclusion: Using a novel transgenic mouse model with a selective and inducible inhibition of NF-κB activation in β-cells, we show that NF-κB blockade confers resistance to cytokine-induced apoptosis *in vitro* and reduces the diabetogenic effect of MLDS *in-vivo*.
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Hypoglycaemia

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Glucose requirements to maintain euglycaemia following afternoon exercise increase abruptly late in the evening in adolescents with type 1 diabetes
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Background and Aims: Regular physical activity benefits individuals with type 1 diabetes. Unfortunately, exercise increases the risk of hypoglycaemia for several hours during recovery. The primary goal of this study was to quantify indirectly the risk of late onset post-exercise hypoglycaemia in adolescents with type 1 diabetes by comparing the differences in the amount of glucose that must be administered to maintain euglycaemia between non-exercised and exercised diabetic subjects.
Materials and Methods: Using a counterbalanced paired design, nine adolescents with type 1 diabetes (5 males: 4 females, age 16 ± 1.8 years, diabetes duration 8.2 ± 4.1 years, HbA1c 7.8 ± 0.8%) were subjected on separate occasions at 16:00 h to 45 minutes of cycling at an intensity of 95% lactate threshold or a 45-minute rest while sitting on the bike. From mid-afternoon of each testing day, a euglycaemic clamp lasting for 15 hours was initiated, with arterialised venous blood glucose levels measured at 15-minute intervals. Insulin was administered intravenously at a constant rate based on usual insulin dose.
Results: The glucose infusion rate necessary to maintain euglycaemia increased abruptly during the first hour post-exercise, returned to basal for the next 5–6 hours, and raised again rapidly 6–7 hours following exercise (2.06 ± 0.42 vs 1.46 ± 0.27 mg/kg/min, exercise vs rest, p<0.05), at a time coincident with the onset of sleep. Plasma insulin levels were similar for both treatments (13.7 ± 0.9 vs 13.8 ± 0.8 mU/l). Moreover, at the onset of recovery from exercise, the levels of the counter-regulatory hormones and free fatty acids (FFA) increased significantly (cortisol, from 270.7 to 557.8 nM, p<0.05; epineprine, from 0.154 to 0.375 nM, p<0.05; norepinephrine, from 1.94 to 5.59 nM, p<0.05; growth hormone, from 5.75 to 67.24 mIU/ L, p<0.05; FFA, from 0.19 to 0.48 mM, p<0.05). However, later during recovery (> 4 hours) there were no significant differences in the levels of these hormones between treatments.
Conclusion: Late afternoon exercise is associated with an early and delayed risk of post-exercise hypoglycaemia as suggested by the higher rates of glucose infusion required to maintain euglycaemia. Mechanisms other than acute changes in the levels of counter-regulatory hormones are likely to explain those findings. Whether a similar amount of ingested carbohydrates would be required to maintain euglycaemia post-exercise remains to be elucidated.

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Patients with type 2 diabetes treated with sulphonylureas develop symptoms of hypoglycaemia but not adrenergic or cognitive responses, at higher glucose levels than insulin treated patients
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Background and Aims: Hypoglycaemia is increasingly recognised as an important clinical problem in type 2 diabetes, with severe episodes affecting patients treated with either tablets or insulin as glucose targets are progressively lowered. An individual's vulnerability to hypoglycaemia will depend upon the relationship between the glucose thresholds for symptoms and cognitive impairment. Previous work suggests that those with tablet treated type 2 diabetes develop symptomatic and counterregulatory responses to hypoglycaemia at higher glucose levels than non-diabetic controls. The effect of antecedent diabetes control is well known but the effect of treatment modality has not been examined. We determined the glucose thresholds for cognitive and counterregulatory hormone release in response to hypoglycaemia in patients with well controlled type 2 diabetes, treated with either insulin or sulphonylureas and in controls.
Materials and Methods: We studied 10 non diabetic controls (C, age 62.8 yrs) and 20 subjects with type 2 diabetes [10 on sulphonylureas (SU) and 10 on insulin (I)] matched for age (SU, I; 67.5 vs 63.4 yrs), weight and HbA_1C

(SU,I; 7.1 vs 7.4%) using stepped hyperinsulinemic hypoglycaemic glucose clamps. We measured symptom responses, counterregulatory hormones and cognitive function [four choice reaction time (4 crt), Stroop word test and Digit Symbol Substitution Test (DSST)] at glucose plateaus of 5, 4, 3.5, 3 and 2.5 mM and calculated glucose thresholds at which there was a significant change from baseline.
Results: Subjects treated with sulphonylureas developed symptoms at significantly higher blood glucose than those treated with insulin (see table) and also had a greater increase in symptom score [13.6(11.3) vs 3.6(6.1), p = 0.02].There were no significant differences between the three groups in thresholds for release of counterregulatory hormones or the magnitude of their peak responses. Glucose thresholds for cognitive dysfunction were also not different.
Conclusion: Our data suggest that the type of treatment may influence the glucose threshold for the onset of hypoglycaemic symptoms in type 2 diabetes despite similar levels of glycaemic control. A higher glucose level for the onset of symptoms in sulphonylurea treated individuals may reduce their vulnerability to severe episodes by alerting them to hypoglycaemia before the onset of cognitive dysfunction.

Glucose thresholds in mM mean(sd)

	Controls	SU	Insulin	
Symptoms	2.93 (0.5)	**3.45 (0.6)**	**2.59 (0.4)**	p= 0.03 (SU v I)
Adrenalin	3.53 (0.5)	3.71 (0.4)	3.42 (0.6)	p=0.38
Glucagon	3.04 (0.9)	3.07 (0.4)	2.86 (0.4)	p=0.83
Cortisol	3.17 (0.4)	3.12 (0.6)	2.85 (0.6)	p=0.26
4 Choice RT	3.39 (0.8)	2.75 (0.5)	2.91 (0.7)	p=0.14
Stroop Colour Word	2.45 (0.1)	2.73 (0.7)	2.51 (0.6)	p=0.47
DSST	2.53 (0.l)	2.78 (0.5)	2.91 (0.8)	p=0.30

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Impact of renin-angiotensin system activity on cognition and symptomatic responses during hypoglycaemia in patients with type 1 diabetes
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Background and Aims: In type 1 diabetes increased risk of severe hypoglycaemia is associated with high renin-angiotensin system (RAS) activity. We tested whether patients with type 1 diabetes and high RAS activity are less able to maintain cognitive function during hypoglycaemia than patients with low RAS activity.
Materials and Methods: From a cohort of 171 patients with type 1 diabetes, 9 patients with high and 9 with low RAS activity (age 40.2 years± 11.5 (mean ± SD); diabetes duration 18.3 years± 8.3; HbA1c 8.3%± 0.86; no significant differences between the two groups) were subjected to hypoglycaemia and euglycaemia in a cross-over study using intravenous (IV) insulin infusion or IV insulin-glucose infusion, respectively. Cognitive function (California Computerized Assessment Package, CalCAP®, a battery of complex reaction time tasks) and hypoglycaemic symptoms were recorded during the experiment.
Results: Despite a similar hypoglycaemic stimulus in the two groups (nadir plasma glucose 2.4 ± 0.3 mmol/l), only the group with high RAS activity showed significant deterioration in cognitive performance in two reaction time tasks requiring the use of working memory (prolongation of reaction times: 116 ± 102 and 133 ± 111 milliseconds in the high RAS activity group (p=0.009 and p=0.032 respectively compared to baseline) versus 2 ± 43 and 55 ± 114 milliseconds in the low group (both NS)). In addition, the high RAS group reported lower increase in autonomic symptom scores during hypoglycemia compared to the low group (score 0.3 ± 4.1 in the high group and 4.6 ± 7.4 in the low group (p=0.04)).
Conclusion: We conclude that in patients with type 1 diabetes high RAS activity is associated with increased cognitive dysfunction and a blunted autonomic symptom response during mild hypoglycaemia compared to patients with low ACE activity. We speculate that cognitive dysfunction and lack of autonomic warning symptoms during hypoglycaemia in patients with high RAS activity may be related to a greater risk of experiencing severe hypoglycaemia in daily life.

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Predisposing factors for hypoglycemia in the intensive care unit
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Background and Aims: The introduction of strict glycemic control in the intensive care unit (ICU) has increased the risk for hypoglycemia. Concern about hypoglycemia may hamper implementation of strict glycemic control. In this study we examined the association between predefined circumstances and the occurence of hypoglycemia in the ICU.
Materials and Methods: All episodes of hypoglycemia (glucose value <45 mg/dl) in our ICU between September 2002 and September 2004 were identified. Presence of predefined circumstances previously associated with hypoglycemia was scored using a patient data management system and in-hospital charts, during the first episode of hypoglycemia and a time-matched moment in control patients. Matching on time was performed to correct for time spent in the ICU before developing hypoglycemia. Data were analyzed using conditional logistic regression analysis. Hazard ratio for death was calculated with Cox regression analysis with time to hypoglycemia entered as a time-dependent co-variate.
Results: Out of 2272 patients, 156 (6.9%) experienced at least one episode of hypoglycemia. Continuous venovenous hemofiltration (CVVH) with bicarbonate-based substitution fluid (Odds Ratio [OR] 14; 95% Confidence Interval [CI] 1.8–106), decreasing nutrition without adjusting insulin infusion (OR 6.6; 95%CI 1.9–23), diabetes mellitus (OR 2.6; 95%CI 1.5–4.7), insulin use (OR 5.3; 95%CI 2.8–11), sepsis (OR 2.2; 95%CI 1.2–4.1) and inotropic or vasopressor drugs use (OR 1.8; 95%CI 1.1–2.9) were associated with hypoglycemia. Simultaneous octreotide and insulin use (OR 6.0; 95%CI 0.72–50) may also be associated with hypoglycemia. Gastric residual during enteral nutrition without adjusting insulin infusion, liver failure, CVVH with lactate-based substitution fluid, diminished glomerular filtration rate, dose diminishment of glucocorticoids or catecholamines and use of beta-blocking agents were not associated with hypoglycemia. Hazard ratio for death was 2.8 (95% CI 1.7–4.6) for patients with hypoglycemia. Adjusting for age, sex and APACHE II score at admission did not materially change OR's.
Conclusion: Use of bicarbonate based substitution fluid during CVVH, not adjusting insulin infusion when decreasing nutrition, a prior diagnosis of diabetes mellitus, sepsis and need for inotropic or vasopressor drugs were found to be associated with hypoglycemia. Simultaneous use of insulin and octreotide may also be associated with hypoglycemia. Occurrence of hypoglycemia is associated with an increased risk for death. However, it seems more likely that hypoglycemia is a consequence of appoaching death than a cause of death.

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Feasibility study on the use of MRS to investigate human brain metabolism in vivo during hypoglycaemia
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Background and Aims: In type 1 diabetes, iatrogenic hypoglycaemia is a common complication of (intensive) insulin therapy that may endanger brain function. Little is known about the effect of hypoglycaemia on cerebral glucose metabolism vis-à-vis glucose delivery and global energy demand. Magnetic Resonance Spectroscopy (MRS) provides several non-invasive methods to investigate these factors in humans in vivo. Here, we tested the feasibility of MRS on the human brain under euglycaemic and hypoglycaemic conditions.
Materials and Methods: Eleven healthy volunteers (9F/2M, 21.9 ± 1.6 years) underwent a two-step hyperinsulinaemic (60–120 $mU \cdot m^{-2} \cdot min^{-1}$) euglycaemic (~5.3 mmol/L; 60 min.) -hypoglycaemic (~2.9 mmol/L; 60 min.) glucose clamp. We measured cerebral glucose content by ^{1}H-MRS (n=5), cerebral glucose uptake and subsequent metabolism by ^{13}C-MRS (n=6), global cerebral energy demand by ^{31}P-MRS (n=4), and cerebral blood flow (CBF) by arterial spin labelling (ASL) (n=3). ^{13}C-MRS was performed using 30% ^{13}C-1-glucose isotopic enrichment, except in one subject in whom 100% ^{13}C-enriched glucose was used during the hypoglycaemic phase of the clamp. All experiments were performed on a sophisticated 3T Siemens Trio MR spectrometer using extended tubing.

Results: Clamp conditions were well tolerated by the participants. Cerebral glucose content, measured by ^{1}H-MRS, averaged 1 µmol/g under euglycaemic conditions, but was undetectable during hypoglycaemia. With plasma ^{13}C isotopic enrichment of 29 ± 1% during euglycaemia and 25 ± 1% during hypoglycaemia, ^{13}C-MRS resulted in spectra of good quality allowing monitoring of brain glucose uptake and time-dependent conversion to metabolites under both glycaemic conditions. Plasma ^{13}C isotopic enrichment averaged 54% during hypoglycaemia in the subject receiving 100% ^{13}C-enriched glucose, which revealed ongoing cerebral glucose uptake and subsequent metabolism under hypoglycaemic conditions. The ^{31}P-MRS experiments also resulted in spectra of good quality. Upon changing the glycaemic condition to hypoglycaemia, the phosphocreatine-ATP ratio remained unaltered, but the resonance of intracellular inorganic phosphate (Pi) showed a slight shift leftwards, equalling an intracellular pH increase from 7.043 to 7.060. CBF measured by ASL was similar during euglycaemia and hypoglycaemia, and averaged 47.2 mL/min/100 mL brain tissue.
Conclusion: Hyperinsulinaemic euglycaemic-hypoglycaemic clamp conditions can be safely applied in humans in a 3T MR magnet. ^{13}C- and ^{31}P-MRS and ASL of the human brain worked sufficiently to allow implementation of these techniques for future studies on hypoglycaemia in human subjects. ^{1}H-MRS lacked sensitivity to detect brain glucose content during hypoglycaemia. Preliminary data indicate that acute hypoglycaemia does not abolish cerebral glucose metabolism and does not alter cerebral blood flow or global cerebral energy demand.
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Functional neuroimaging study using low resolution electromagnetic tomography (LORETA) for neurophysiological assessment in type 1 diabetic patients with history of recurrent severe hypoglycaemia
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Background and Aims: History of recurrent severe hypoglycaemia with unconsciousness was recognized as predictive for further such events in Type 1 diabetic patients. It is associated with a more rapid decrease in vigilance (slowing of brain function) during hypoglycaemia in comparison to patients without history of such events. Diminished vigilance was found in recurrent severe hypoglycaemia also in non-hypoglycaemic state. Our aims were (1) to use a three-dimensional EEG tomography for spatial description of neurophysiological characteristics in representative groups of Type 1 diabetic patients *with* and *without* previous recurrent severe hypoglycaemia and (2) to compare them with non-diabetic controls.
Materials and Methods: A vigilance-controlled EEG was performed in non-hypoglycaemic state (blood glucose 4.0–10.0 mmol/l) in a group of 13 Type 1 diabetic patients *with* a history of recurrent severe hypoglycaemia and compared to that of 14 Type 1 diabetic patients *without* such history, matched for HbA_{1c}, age and gender, and to age- and gender-matched *non-diabetic* controls. Low-resolution electromagnetic tomography (LORETA) was computed from spectrally analyzed EEG data, and differences between groups were displayed as digitized threedimensional statistical parametric maps.
Results: When compared to non-diabetic controls, hypoglycaemia patients demonstrated increase of theta in left inferior frontal gyrus, frontal lobe (frontal associative cortex) and of alpha in the left middle and superior frontal gyri, frontal lobe (up to $p<0.01$). Beta decrease was present temporoparietal. Patients *without* hypoglycaemia history compared to healthy controls demonstrated only minor theta decrease parietooccipital. Patients *with* history of hypoglycaemia compared with patients *without* demonstrated a delta increase in left superior temporal gyrus, temporal lobe and theta increase in medial frontal gyrus, frontal lobe.
Conclusion: Deceleration in hypoglycaemia patients was most remarkable in associative pre frontal cortex of the dominant hemisphere. It resembles *attention deficit syndrome* and its spatial distribution links to brain areas necessary for recognition.

OP 18

Inflammation and type 2 diabetes: basic mechanisms

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The insulin sensitive glucose transporter GLUT4 is a molecular target of the IKK and NF-kB pathway
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Background and Aims: Inflammatory signaling pathway has been suggested in the pathogenesis of insulin resistance and Type 2 Diabetes Mellitus (DM2) by inhibiting IRS1-PI3 kinase. Inhibiting IkappaB kinase (IKK) activity was shown to increase insulin sensitivity. Therefore, we examined whether the insulin-responsive glucose transporter (GLUT4) is a direct molecular target for IKK- NF-kB transcriptional complex and its relevance to diabetes.
Materials and Methods: C2C12 myotubes and HEK-293 cell-lines were transiently co-transfected with luciferase-conjugated GLUT4 promoter reporter and expression vectors for either IKK or NF-kB subunits.
Results: Over-expression of IKK (IKKalpha and IKKbeta) dose-dependently repressed GLUT4 promoter transcriptional activity to 60% of basal level. This effect was abolished in presence of salicylates (10 mM, 48 hr). While over-expression of wt IKK-α alone increased GLUT4 promoter activity to ~125%, and ~200% of its basal levels, in HEK-293 and C2C12 cells, respectively, over-expression of IKK-β decreased GLUT4 promoter activity to ~60% and ~90% of its basal levels, in HEK-293 and in C2C12 cells, respectively. Thus these two subunits have diverged function. Incubating the transfected cells in the presence of 10 mM sodium salicylate for 48 hours abolished the inhibitory effect of IKK-β in HEK-293 cells and increased IKK-α activation of GLUT4 gene transcription. IKK-β mutants SS177/181AA and K44M that lead to p65 cytoplasm sequestration exhibited no transcriptional effect on the GLUT4 promoter. However, the constitutively active mutant SS177/181EE - that induces p65 translocation into the nucleus, suppressed the transcription activity slightly more than the wild type.
Co-expression of NF-kB p50/p65 heterodimer dose-dependently decreased GLUT4 promoter transcriptional activity by 70% and 25% in HEK and C2C12 myotubes. In contrast, co-expression of p50 subunit increased the GLUT4 promoter transcriptional activity by ~200%, while p65 alone repressed it by ~50%. 5'-deletion analysis revealed specific DNA regions on the GLUT4 promoter that might mediate the different NF-kB effects. Measuring p50 and p65 protein levels in muscle biopsies obtained from diabetic and normal control patients showed a marked reduction of p50 subunits to ~50% of control level but no significant change in and p65 levels. Using specific siRNA, p65 protein subunit was reduced to ~30% compared to control level followed by 150% elevation in cellular GLUT4 protein level.
Conclusion: Our data suggest that NF-kB subunits are regulators of the GLUT4 gene transcription. Since p65 suppression resulted in significant increase of cellular GLUT4 protein level, modulation of IKK- NF-kB pathway is a potential target for type 2 diabetes therapy.
Support: L.R Diamond Fund

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Differential regulation and activation of the c-Jun N terminal kinase (JNK) in human abdominal adipose tissue
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Central obesity is strongly associated with insulin resistance and implicated in the development of type 2 diabetes mellitus (T2DM) but the intracellular mechanisms involved in mediating this link remain unclear. Recently c-Jun N terminal kinase (JNK), a mitogen activated protein kinase (MAPK), has been shown to play a central role in obesity and insulin resistance by interfering with insulin action. Our previous studies demonstrated that human adipose tissue expresses total JNK (JNK1/JNK2) and its active forms. Further, total JNK is expressed in a depot specific manner with increased expression in "central fat" compared to thigh fat. However this was not found to be correlated with adiposity. Therefore in this study we proposed to investigate: a) the JNK expression in abdominal subcutaneous (AbdSc) and omental (Om) adipose tissue, b) the *in vitro* effect of a known

JNK mediator: tumour necrosis factor-alpha (TNF-α) and its antagonist in AbdSc adipocytes and c) the potential mechanisms of action of JNK in inflammation in AbdSc and Om fat. Our findings demonstrated that total protein expression of JNK varied according to the fat depot with increased expression in the Om compared with AbdSc depots ($p<0.01$); this was unrelated to the presence of macrophages as assessed in our previous studies. Furthermore, phosphorylated forms of JNK1 and JNK2 were increased compared to AbdSc compared with Om adipose tissue (0.74, 0.49 fold increase respectively, $p<0.01$). In addition, we assessed the relative activity of JNK1 and JNK2 compared with total JNK activity for each depot. We determined that Om adipose tissue contained the lowest activity of JNK1 and JNK2 compared with AbdSc adipose tissue ($p<0.01$). Whilst assessment of the *in vitro* effect of TNF-α in the presence or absence of TNF-α antagonist on subcutaneous abdominal adipocytes indicated that both JNK1 and JNK2 activity did not appear to be altered. NEMO (also known as IKKγ), IκBα and its phosphorylated (IκBα-P) form were shown to be upregulated in Om compared to AbdSc ($p<0.01$). In addition, we also observed a significant increase in IκBα -P expression in overweight and obese compared to lean ($p<0.05$). Therefore suggesting increased pathogenic release of adipocytokines in Om fat whilst this appears to be unrelated to c-Jun kinase expression in this depot. In summary, this study clearly demonstrates differential JNK expression and functional activity in human abdominal adipose. Furthermore, we have shown that an established JNK modulator, TNF-α failed to regulate JNK activity in isolated human AbdSc adipocytes. JNK activity appeared unrelated to NEMO and IκBα activity in Om fat indicating that insulin resistance in this particular fat depot is unrelated to c-Jun N terminal kinase. In conclusion, these data suggest that JNK activity may not have a direct effect on insulin resistance in Om fat. Therefore indicating that mediators of JNK, other than TNF-α may affect JNK activity to contribute to insulin resistance and the progression in the pathogenesis of T2DM.

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CRP increases lipoprotein lipase expression by macrophages

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Background and Aims: C-reactive protein (CRP), an inflammatory biomarker and a strong predictor of cardiovascular events, is increased in patients with type 2 diabetes. Besides being a risk marker, CRP may act as a mediator of atherogenesis by directly inducing atherothrombotic effects on vascular cells. In monocytes/macrophages, these effects include cell activation with increased cytokine and chemokine expression, enhanced monocyte-endothelium interaction and increased foam cell formation. The aim of this study was to examine whether CRP could affect the expression of macrophage lipoprotein lipase (LPL), a proatherogenic molecule upregulated in type 2 diabetes.
Materials and Methods: Cultured J774 murine macrophages or totally differentiated monocyte-derived macrophages isolated from healthy subjects, were incubated for different time periods (1–24 hours) with increasing concentrations of recombinant CRP (1–25 μg/ml). At the end of these incubation periods, LPL protein and gene expression were measured by Western and Northern blot analysis, respectively.
Results: Incubation of J774 macrophages with 3 μg/ml CRP significantly increased macrophage LPL protein and gene expression (LPL protein levels [% increase over control values]: 221 ± 49, $P<0.05$), (LPL mRNA expression [% increase over control values]: 173 ± 17, $P<0.01$). When used at the same concentration, CRP also enhanced LPL protein expression in human macrophages. In contrast, incubation of macrophages with high CRP concentrations (≥10 μg/ml) significantly decreased LPL protein and gene expression.
Conclusion: Taken together, these data show for the first time that CRP, at a concentration associated with increased cardiovascular risk, enhances macrophage LPL expression both at the gene and protein levels. Given the predominant role of macrophage LPL in the development of atherosclerosis, these results suggest a new mechanism by which CRP may directly promote atherogenesis, that of inducing macrophage LPL.
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Activation of peripheral blood CD14$^+$ monocytes occurs in diabetes mellitus

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Background and Aims: Atherosclerosis is a major complication of diabetes. More and more evidence suggests that an inflammatory process is important in the development of atherosclerosis. Lipid uptake through the scavenger receptor, CD36, monocyte-derived macrophages in the vessel wall leading to foam cell formation is an important initial step in atheroma formation. Therefore we investigated the state of the peripheral blood monocyte, from well controlled (WCD) and poorly controlled (PCD) diabetic patients and non diabetic controls (NDC), with respect to scavenger receptor (CD36) cell surface expression and gene expression. Gene expression of atherosclerotic markers such as MCP-1, ICAM-1, and VCAM-1 were also studied.
Materials and Methods: CD14$^+$ monocytes were isolated from peripheral blood of Type 1 and Type 2 diabetic patients with good (HbA1c < 7.0%) or poor (HbA1c > 9.4%) glycaemic control and from a group of non-diabetic subjects using anti-CD14 magnetic microbeads. CD36 cell surface expression was assessed by flow cytometry. OxLDL uptake was measured after labelling the oxLDL with DiI. mRNA expression of CD36, CD68 (a marker of the mature macrophage), MCP-1, ICAM-1, and VCAM-1 were examined by real time RT-PCR. Adhesion of circulating monocytes to endothelial monolayers was assessed after labelling the monocytes fluorescently with CMFDA.
Results: The percentage of CD36 positive cells was higher in the PCD (64%) than in the WCD (37%) and in the NDC (4%). CD36 mRNA was increased by 130% and 50% in PCD and WCD, respectively compared to NDC; CD68 mRNA was significantly increased only in the PCD. The increase in CD36 cell surface expression was associated with an increase in oxLDL uptake in PCD (+ 433%) and WCD (+ 243%). Additionally, MCP-1 was significantly increased in both groups of diabetic subjects, by 278% and 237% in PCD and WCD, respectively, whereas there was no significant increase in ICAM-1 and VCAM-1. Monocyte attachment to normal endothelial cells was increased in both diabetic subject groups but was significant only in PCD (+ 259%).
Conclusions: Subjects with poorly controlled diabetes are at higher risk for the vascular complications of diabetes than those who are well controlled. Activation of scavenger receptors on monocytes in the peripheral blood may be an early clinical marker for accelerated atherosclerosis.

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High glucose and homocysteine synergistically affect MMP-TIMP pattern but not TGFβ expression in human fibroblasts

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Background and Aims: Atherosclerosis is particularly aggressive in patients with diabetes. Hyperhomocysteinaemia (Hcy) causes endothelial damage, oxidative stress and cytokine secretion: its atherogenic effect could be mediated by an enhanced inflammatory response. Extracellular matrix degradation and remodelling, which contributes to the vulnerability of the atherosclerotic lesion and promotes plaque rupture, is mainly due to matrix metalloproteinases (MMPs), known to be also involved in the pathogenesis of restenosis and myocardial infarction. Their activity is modulated by a family of endogenous tissue inhibitors (TIMPs). Alterations in the balance between MMPs and TIMPs might have a profound impact on the atherosclerotic process. Fibroblasts are the major contributor to collagen biosynthesis and participate in plaque remodelling via expression and release of MMPs 2 and 9. In order to explore the role of Hcy in cellular pathways involved in plaque growth and stability in diabetes, we studied the effect of Hcy in human primary fibroblasts grown in the presence of normal or high glucose concentrations.
Materials and Methods: Fibroblasts from skin specimens of 5 normal subjects, grown at 5.5 or 22 mM glucose were incubated with 10 and 100 μM Hcy for 6 hours. MMP2, MMP9 and TIMP1 proteins were determined by Western Blot analysis. The activity of MMP2 and MMP9 in the supernatants was assessed by zymography. Interleukin-6 (IL6) release was measured by ELISA; TGFβ expression was assessed by real-time polymerase chain reaction.
Results: Hcy increased the expression of both MMP2 and MMP9 in a dose-dependent fashion, and this effect was more pronounced in cells grown at high glucose (for β-actin=100 arbitrary units, MMP2: 70 ± 5 at baseline, 75 ± 8 with 10 μM Hcy and 80 ± 13 with 100 μM Hcy at 5.5 mM glucose *vs*

70 ± 13, 103 ± 10 and 124 ± 16 at 22 mM glucose, both p<0.01 *vs* 5.5 mM glucose; MMP9: 47 ± 7 at baseline, 71 ± 10 with 10 μM Hcy and 116 ± 17 with 100 μM Hcy at 5.5 mM glucose *vs* 87 ± 10, 119 ± 20 and 136 ± 13 at 22 mM glucose; both p<0.01 *vs* 5.5 mM glucose). Conversely, TIMP1 expression was reduced by Hcy both in both conditions, the effect being more pronounced in high glucose (5.5 mM: 102 ± 6 at baseline, 103 ± 10 with 10 μM Hcy and 80 ± 5 with 100 μM Hcy; 22 mM: 140 ± 9 at baseline, 122 ± 13 with 10 μM Hcy and 61 ± 7 with 100 μM Hcy, p<0.01 *vs* 5.5 mM glucose for both). In keeping with the Hcy-induced increase in MMP2 and MMP9 expression, their enzymatic activity was enhanced (MMP2: +95% at 5.5 and +169% at 22 mM glucose; MMP9: +144% at 5.5 and +152% at 22 mM glucose after stimulation with 100 μM Hcy). Hcy 100 μM also stimulated IL6 release (from 1698 ± 106 to 1867 ± 113 at 5.5 mM *vs* from 2628 ± 132 to 2876 ± 108 pg/ml/5 x 10^5 cells at 22 mM, both p<0.05 vs baseline and p<0.001 for Δs between 5.5 and 22 mM). Upon co-incubating cells with 100 μM Hcy for 6 hours, TGFβ mRNA expression (after normalisation for a housekeeping gene) rose from 1.04 ± 0.1 to 1.23 ± 0.1 at 5.5 mM glucose, p<0.02, while no significant differences were observed at 22 mM glucose (from 1.48 ± 0.2 to 1.35 ± 0.4, p=ns).
Conclusion: Homocysteine upregulates the MMP-TIMP pathway and IL6 release, the effect being stronger in the presence of high glucose. Through these actions, homocysteine may contribute to the increased atherogenesis observed in diabetic patients in poor metabolic control.

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High glucose treatment upregulates TNF-α expression in human monocytes of healthy subjects but not in diabetic patients
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Background and Aims: Hyperglycaemia and chronic inflammation have been implicated in a number of diabetic complications. A likely common denominator of all these abnormalities could be the dysfunction of the cell-mediated immune system. Mononuclear cells (PBMNCs) may provide and easy and representative view of the overall inflammation status of the body. The pro-inflammatory tumor necrosis factor alpha system (TNFα and its receptors TNFR1 and TNFR2) plays an important role in the pathogenesis of diabetes as an inhibitor of insulin action. We have analysed, in primary cultures of mononuclear cells (monocytes and lymphocytes fractionated by adherence) from healthy subjects and from type 2 diabetic patients, the response of the TNFα system under inflammatory and hyperglycaemic conditions
Materials and Methods: The study group comprises 16 patients, 8 healthy controls and 8 recently diagnosed type 2 diabetic patients (basal glucose 6.66+ 0.68 mM, HbA1c 5,68 ± 0.33%) treated only with diet. PBMCs were isolated from 40 ml of blood by density centrifugation on Histopaque 1077. The cells were resuspended in RPMI-1640 supplemented medium and incubated for 1 hour a 37 °C in 5% CO_2 to allow for monocyte attachment. The non-adherent fraction (lymphocytes) was also used in the experiment. The attached monocytes and non-adherent fraction was treated with the following conditions: 1 μg/ml LPS (lipopolysaccharide), 10 mM Glucose and 20 mM Glucose at 37 °C 5% CO_2 for 10 h. RNA was extracted from cell cultures by using RNAsy® Mini Kit, following manufacturer's instructions Quantification of the mRNA coding for TNF-α, TNFR1, TNFR2, CD14 and β-actin was performed using LightCycler technology. Plasma and culture medium secreted TNF-α was measured by ELISA.
Results: High glucose treatment produced 2.1 fold up-regulation of TNFα mRNA level in monocytes of healthy subjects (p<0.018), but failed to elicit a response on cultured monocytes from type 2 diabetic patients. On the other hand, TNFR2 expression was up regulated only in lymphocytes from type 2 diabetic patients (p=0,036). LPS treatment resulted in a dramatic increase of TNFα mRNA expression (20 to 30 fold, p<0.012) and protein expression in monocytes from both groups (although the increase was always higher in type 2 diabetic patients).
Conclusion: Our finding indicate that TNFα system in mononuclear cells from type 2 diabetic patients have a different behaviour to hyperglycaemia and to LPS stimulus. This argues in favour of a predisposition to chronic sub-clinical inflammation before a marked hyperglycaemia developed.
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OP 19
Oral agents, experimental aspects

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AICA-riboside is a potent vasodilator and decreases blood glucose levels in humans
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Background and Aims: Exercise and ischemia stimulate glucose uptake in skeletal muscle by translocation of glucose transporters (GLUT4). This action is independent of insulin signalling and seems to be mediated by AMP activated protein kinase (AMPK). As such, AMPK is a potential pharmacological target for the treatment of type 2 diabetes. AMPK can be stimulated by 5-aminoimidazole-4-carboxamide-riboside (AICA-riboside). Animal studies have shown that AICA-riboside stimulates glucose uptake and improves insulin sensitivity. So far, no studies in humans have been reported. Apart from the metabolic effects, AICA-riboside may have vasodilator properties because it is an analogue of the endogenous nucleoside *adenosine*. In the present study, we investigated the effect of AICA-riboside on forearm skeletal muscle blood flow (FBF) and forearm glucose uptake (FGU) in healthy subjects. In addition, we measured the effects of AICA-riboside after inhibition of the *endogenous nucleoside transporter* by dipyridamole.
Materials and Methods: AICA-riboside was infused into the brachial *artery* for 110 minutes in a dose of 1, 2 or 4 mg/min per dl of forearm tissue, in three groups of 6 healthy subjects (mean age 21.3 ± 2.0, BMI 21.1 ± 2.0 kg/m^2). Four subjects received 8 mg/dl/min. FBF was measured by plethysmography. Arterial and deep forearm venous plasma glucose concentrations were measured for calculation of FGU. In 5 subjects, AICA-riboside (1 mg/min/dl) infusion was repeated, but now in the presence of dipyridamole (7.4 nmol/min/dl).
Results: AICA-riboside had a potent, dose-dependent, vasodilator effect at the infusion side (FBF from 2.2 ± 0.3 to 4.9 ± 0.7, from 2.3 ± 0.3 to 7.3 ± 1.8 and from 2.4 ± 0.5 to 8.3 ± 2.3 ml/min/dl for 1, 2 and 4 mg/min/dl resp., all P<0.05). After discontinuation of AICA-riboside, FBF returned to baseline values. Dipyridamole did not change the vasodilator effect of AICA-riboside.
AICA-riboside decreased arterial (= systemic) plasma glucose from 4.8 ± 0.1 to 4.4 ± 0.1 mmol/l without a clear dose-response relation (pooled data, n=18, P<0.05). After discontinuation of AICA-riboside, arterial plasma glucose levels returned to 4.6 ± 0.1 mmol/L (n=18, P<0.05 versus end of AICA-riboside infusion). Intrabrachial AICA-riboside infusion had no effect on FGU. AICA-riboside in a dose of 8 mg/min/dl appeared to have a more pronounced effect on systemic plasma glucose level (preliminary data, n=4, glucose from 5.0 ±.0.2 to 4.1 ± 0.3 mmol/l, P<0.05, returning to 4.7 ± 0.2 mmol/L after discontinuation of infusion).
Conclusion: AICA-riboside is a potent, dose-dependent vasodilator in the skeletal muscle vascular bed of healthy volunteers. Dipyridamole did not affect the vasodilator response to AICA-riboside, arguing against a role of the *endogenous nucleoside transporter* in this response. AICA-riboside lowered systemic plasma glucose levels, without an effect on forearm skeletal muscle glucose uptake. This observation suggests that other mechanisms, for example inhibition of liver glucose production, are involved.
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GW501516, a specific agonist of PPARδ, has acute PPAR-independent effects on mitochondrial function
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Background and Aims: GW501516 (GW) is a specific and potent agonist of peroxisome proliferator-activated receptor-δ (PPARδ), which has been shown to ameliorate hyperglycaemia, insulin resistance, and hyperlipidaemia in animal models of type 2 diabetes. We have shown previously that GW distinctly increases fatty acid oxidation and reduces glucose utilisation in isolated rat skeletal muscle, which is obviously mediated by a PPARδ-dependent genomic mechanism of action. In the course of our experiments, however, we noted that GW had acute effects on muscle fuel meta-

bolism which could not be explained by this mechanism. The present study was to examine whether GW has direct PPAR-independent actions on mitochondrial function that could contribute to its effects on fuel metabolism, because such actions characterise agonists of other PPAR subtypes.
Materials and Methods: Mitochondria were isolated from rat liver and oxygen consumption was measured by a Clark-type oxygen electrode in the absence or presence of various concentrations of GW. Results shown in this abstract refer to glutamate/malate-respiring mitochondria, but the effects of GW were essentially identical in mitochondria provided with succinate. Furthermore, insulin-stimulated rates of glucose metabolism were measured in freshly isolated rat soleus muscle after short-term (0.5 h) or long-term (24 h) pretreatment with GW *in vitro*. All data are given as % change vs control (i. e. identical conditions without GW).
Results: In isolated mitochondria, GW dose-dependently reduced state 3 respiration (10 µmol/l GW, –4 ± 2%, ns; 30 µmol/l GW, –8 ± 2%, p=0.02; 100 µmol/l GW, –23 ± 2%, p<0.001), whereas it increased state 4 respiration (10 µmol/l GW, –2 ± 1%, ns; 30 µmol/l GW, +1 ± 2%, ns; 100 µmol/l GW, +15 ± 2%, p=0.002). Accordingly, GW markedly reduced respiratory control (i. e. the ratio of state 3/state 4 respiration; 10 µmol/l GW, –2 ± 2%, ns; 30 µmol/l GW, –9 ± 1%, p=0.001; 100 µmol/l GW, –33 ± 1%, p<0.001) as well as the ratio of ADP consumption/oxygen consumption (10 µmol/l GW, –1 ± 1%, ns; 30 µmol/l GW, –2 ± 1%, p=0.06, ns; 100 µmol/l GW, –10 ± 1%, p<0.001). These results strongly indicate a direct uncoupling effect of GW on mitochondria. Increased uncoupled respiration explains a GW-induced elevation of palmitate oxidation as seen after 0.5 h of pretreatment in isolated rat muscle, which is not blocked by cycloheximide and, hence, can not be attributed to a PPARδ-dependent genomic mechanism (30 µmol/l GW alone, +29 ± 6%, p<0.001; 30 µmol/l GW in the presence of 1 mg/l cycloheximide, +42 ± 12%, p<0.006). With regard to the long-term actions of GW (24 h), we observed a U-shaped dose response curve for glucose oxidation, which obviously resulted from genomic PPARδ-mediated inhibition of glucose oxidation at lower concentrations and enhanced glucose oxidation due to PPAR-independent uncoupling at higher concentrations (0.01 µmol/l GW, –11 ± 3%; 0.1 µmol/l GW, –31 ± 7%; 1 µmol/l GW, –46 ± 8%; 3 µmol/l GW, –25 ± 7%; 10 µmol/l GW, –27 ± 8%; p<0.01 each; 30 µmol/l GW, –7 ± 6%, ns).
Conclusion: Like other PPAR agonists, GW has direct PPAR-independent effects on mitochondrial function. PPAR-independent uncoupling could contribute to increased energy expenditure as seen with oral GW-treatment *in vivo*.
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Mechanism of action studies with novel thiazolidinedione BLX-1002 with no PPAR γ affinity
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Background and Aims: BLX-1002 is a novel anti-hyperglycemic thiazolidinedione (TZD) compound (MW<500) developed by the "Rational Drug Design Approach" (RDDA). Although, it contains both tyrosine and TZD moieties, it does not show affinity to any PPAR-α, γ or δ receptors. This was demonstrated earlier by various assays like adipogenesis, transactivation, gene expression (aP2, Adiponectin) and with radioligand binding assays. Interestingly, the compound is very effective in insulin resistant *db/db* animals, at a dose of 12.5 mg/kg bodyweight (daily single oral dose) and was able to reduce blood glucose levels more than 40% within 4–5 days of treatment. BLX-1002 lowers serum triglycerides, free, fatty acid and total cholesterol levels compared to vehicle treated animals. BLX-1002 also lowers blood glucose levels in non obese diabetic (NOD) and streptozotocin induced diabetic mice. It has strong inhibitory effect on lipopolysacharide (LPS) induced serum TNF- (72% ↓) and IL-6 (42% ↓) in animals. Unlike other two marketed TZDs, it does not show any liver, heart, body weight gain in different animal models. Because of its efficacy, in both type-1 and type-2 diabetes animal models, a series of mechanistic studies were carried out to elucidate the mechanism of action of this novel TZD BLX-1002.
Materials and Methods; Results: BLX-1002 was tested in several purified key enzymes in insulin signaling pathway, like Insulin Receptor Tyrosine Kinase, PTP-1B, PKBα (Akt) and in GSK-3β enzymes and 10 µM concentration of BLX-1002 had no effect (0–6%) in all of these enzymes. Different receptor- radioligand binding assays were carried out with Adrenergic-β3, GLP-1, Insulin Receptor and voltage-insensitive K_{ATP} channel receptors and BLX-1002 failed to show any affinity (3–9%) towards those receptors at 10 µM concentrations. In other target enzyme assays, it has no inhibitory effect on Fructose-1, 6 bisphosphatase, Alpha-D-Glucosidase, Glycogen phosphorylase and DPP-IV enzymes, but strongly inhibited Aldose Reductase, a key enzyme for polyol pathway, which plays a crucial role in diabetic complications. Purified rat lens aldose reductase enzyme is dose dependently inhibited by BLX-1002 (IC_{50} 4.05 µM), whereas Quercitrin, the positive control showed strong inhibition (IC_{50} 0.35 µM).
In a different set of experiments it was also observed that like Metformin, BLX-1002 activates the AMP-activated protein kinase (AMPK) in human liver cells. Metformin at 2 mM dose activates AMPK 11% over basal whereas BLX-1002 (0.2 mM) in the same system increased 30% phosphoactivation over basal. BLX-1002 may have effects on other AMPK regulated downstream events. It was also found in LPS induced mouse macrophage system BLX-1002 inhibited iNOS (inducible nitric oxide synthatase) expression which is also known to govern by AMPK enzyme.
Conclusion: Taken together, BLX-1002 is a novel thiazolidinedione antidiabetic compound with no affinity to any PPAR receptors, activates AMPK in a different cell types and inhibits aldose reductase enzyme. In different Phase-I clinical trials it was demonstrated that BLX-1002 is safe and well tolerated in both normal and diabetic subjects. Thus, BLX-1002 is a novel, Non-PPAR-Thiazolidinedione compound, with no issues of weight gain, fluid retentions, hepatotoxicity and can be useful for the treatment of insulin resistance and associated complications.

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Effect of rosiglitazone on matrix components and cytokine secretion in human fibroblasts
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Background and Aims: PPARs are key players in lipid and glucose metabolism and are implicated in metabolic disorders predisposing to atherosclerosis, such as dyslipidaemia and diabetes. We evaluated the effect of rosiglitazone (Rosi), a PPARγ agonist, on the expression and release of some proliferative and inflammatory cytokines in human fibroblasts, a crucial cell in the growth and remodeling of the atherosclerotic plaque.
Materials and Methods: Cells from skin specimens of five normal subjects, grown at 5.5 mM glucose concentration, were incubated with Rosi at two concentrations (2.1 and 4.2 M) for 6 hours. TGFβ and IL6 expression and release were aestimated by realtime-PCR and ELISA, respectively, as well as the levels of extracellular matrix components, laminin and fibronectin. In addition, we tested the effect of the PPAR antagonist, Sr202 in modulating or reversing the above-described effects.
Results: After normalization for an housekeeping gene, the relative mRNA expression (RelmRNA) for TGFβ was 3 after PMA (a PKC stimulator) and 4 after Rosi; the combination of both stimuli induced a RelmRNA of 8. Sr202 completely abolished the effect of Rosi without interfering with PMA action. In the same cells, laminin and fibronectin production was increased by Rosi (from 69 ± 10 to 99 ± 12 ng/ml/1.5 x 10^5cells, p=0.003, and from 1180 ± 163 to 1444 ± 121 ng/ml/1.5 x 10^5cells, p=0.02, respectively); this effect too was completely reversed by Sr202 (back to 74 ± 11 for laminin and 1211 ± 128 ng/ml/1.5 x 10^5cells for fibronectin). Rosi *per se* had a weak effect on IL6 expression (RelmRNA: 1 vs 1.4), while decreasing by ~50% the increase induced by PMA (from 4 to 2 RelmRNA). As a result, Rosi was able to maintain IL6 release at similar levels as the unstimulated state (722 ± 34 vs 654 ± 61 pg/ml/1.5 x 10^5cells, p=ns) and to partially reverse the effect of PMA (from 1096 ± 30 with PMA to 696 ± 22 pg/ml/1.5 x 10^5cells with Rosi+PMA, p<0.001). In the absence of Rosi, Sr202 did not change the effect of PMA (PMA+Sr202: 1662 ± 148 pg/ml/1.5 x 10^5cells).
Conclusion: In human fibroblasts Rosi promotes matrix production via enhanced TGFβ expression and reduces PMA-induced IL6 expression and release, thus suggesting a possible role of this compound in the treatment of vascular complications of diabetes.

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Uptake, metabolism and inhibitory potency on gluconeogenesis of MB06322, a prodrug of a specific fructose 1,6-bisphosphatase inhibitor, in primary human hepatocytes
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Background and Aims: Increased hepatic glucose production (HGP) resulting from dysregulated gluconeogenesis (GNG) is a hallmark of type 2 diabetes (T2DM) and an attractive site for therapeutic intervention. MB06322 is an uncharged, bis-amidate prodrug of MB05032, a rationally-designed, specific inhibitor (IC_{50} 16 nM) of a key regulatory enzyme of GNG, fructose 1,6-bisphosphatase (FBPase). MB06322 (CS-917) has been shown to lower blood glucose in acute and subchronic evaluations in diabetic animal models as well as in a Phase IIa trial in T2DM patients. Efficacy in rodents has been attributed to inhibition of GNG and HGP using tracer techniques;

however, this has not been assessed in a human model. The purpose of these studies was to verify the conversion of MB06322 to MB05032 in human hepatocytes as well as its inhibitory potency on GNG relative to metformin. Metformin has been demonstrated to lower blood glucose in patients in part by inhibiting GNG.
Materials and Methods: MB06322 was incubated with cryopreserved human hepatocytes (1.5×10^6 cells/mL) under physiological conditions for 2 hours. Aliquots of the cell suspension were removed at various time points, extracted, and analyzed by reverse phase HPLC to assess prodrug uptake and metabolism. Gluconeogenesis was assessed in fresh, primary human hepatocytes. After equilibration and suspension in glucose-free Krebs-bicarbonate buffer, test agents were added at a range of concentrations, followed by gluconeogenic substrates [0.2 mM pyruvate and 2 mM ^{14}C lactate (5 μCi/mmol)]. Reactions were stopped by centrifugation. Labeled glucose in the supernatants was quantified by liquid scintillation counting following its separation from charged intermediates by batch anion exchange chromatography.
Results: MB06322 was rapidly taken up and metabolized ($t_{1/2}$ <1 min) in cryopreserved human hepatocytes to a metabolite that co-migrated with a known mono-amidate intermediate, MB06633. Conversion of MB06633 to MB05032 occurred at a slower rate (0.4 nmoles/million cells/min). In separate studies in fresh human hepatocytes, MB06322, MB06633, and MB05032 inhibited GNG with IC_{50} values of 0.12, 16, and 52 μM, respectively. Metformin (IC_{50} = 280 μM) was >2000-fold less potent than MB06322 in the assays. Cellular viability, as assessed by Trypan blue exclusion, was not compromised by any of the treatments.
Conclusion: MB06322 is converted to MB05032, the active FBPase inhibitor, in human hepatocytes via a 2-step mechanism. Cleavage of the 2nd phosphoramide bond of MB06322 appears to be the rate-limiting step in its activation. MB06322 (IC_{50} 0.12 μM) was a potent inhibitor of de novo glucose production in these cells, while MB06633, an intermediate in prodrug activation, and MB05032 were significantly less potent. As MB06633 and MB05032 are both charged at physiological pH, the reduced potency observed is likely a consequence of a slower rate of cellular penetration. Delivery of MB05032 as an uncharged prodrug is thus not only important for enhancing oral bioavailability as demonstrated in other studies, but also to facilitate uptake in hepatocytes and inhibition of GNG. The increased potency of MB06322 relative to metformin suggests that MB06322, a novel and direct inhibitor of GNG, may provide an effective treatment of T2DM, however, its safety and efficacy has not been proven in human clinical trials.

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Gliclazide protects isolated human islets from apoptosis induced by intermittent high glucose: the role of oxidative stress

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Background and Aims: Concerns have been raised regarding the loss of beta-cell function and mass following long-term sulphonylurea treatment of type 2 diabetic patients. Studies with human islets to more directly investigate on such issues are scarse and have provided inconsistent results.
Materials and Methods: In the present study, human islets (HI) were isolated from the pancreas of 7 multiorgan donors (age: 57.6 ± 18.7 yrs; M/F: 5/2; BMI: 24.3 ± 3.4 Kg/m^2) and then cultured for 5 days in continuous normal (NG, 5.5 mmol/l) glucose medium, or normal and high (HG, 16.7 mmol/l) glucose media alternating every 24 h, either with or without the addition of therapeutical concentration (10 μmol/l) of glibenclamide (GLIB) or gliclazide (GLICL).
Results: At NG incubation and in the absence of sulphonylureas, insulin release (IR), expressed as stimulation index (SI, i. e. the ratio of acute IR at 16.7 mmol/l glucose over acute IR at 3.3 mmol/l glucose), was 2.2 ± 0.9 (control value, Ctrl). The presence of GLIB (62.3 ± 20.9% of Ctrl, $p<0.05$), but not that of GLICL (77.7 ± 21.0% of Ctrl, $p=0.3$), induced a significant reduction of SI. HG incubation determined a decrease of SI (63.1 ± 10.6% of Ctrl, $p<0.01$), which was not further affected by GLIB (53.9 ± 20.0%) or GLICL (62.9 ± 18.9%). Beta-cell apoptosis, as assessed by electron microscopy, was 0.5 ± 0.1% in HI at NG incubation, and increased to 11.7 ± 4.2% ($p<0.01$) at HG, with a further increase with GLIB (20.7 ± 8.5%). However, incubation with GLICL resulted in a significant reduction of apoptosis (5.7 ± 3.8%, $p<0.05$ vs HG alone). This positive effect of GLICL was associated with reduced oxidative stress (nitrotyrosine concentration, ng/ml: 5.3 ± 2.2 at NG; 14.3 ± 7.6 at HG, $p<0.01$ vs NG; 8.4 ± 4.9 at HG and GLIB, NS vs HG; 5.9 ± 3.3 at HG and GLICL, $p<0.05$ vs HG). In addition, mitochondria volume density (ml%), which increased from 4.0 ± 0.5 at NG to 7.6 ± 0.5 at HG ($p<0.01$), returned to 4.4 ± 0.7 at HG and GLICL ($p<0.05$ vs HG).
Conclusion: At the present experimental conditions, prolonged in-vitro exposure to gliclazide did not significantly damage isolated human islet insulin secretory function. Most importantly, gliclazide was able to protect human beta-cells from apoptosis induced by intermittent high glucose, an effect which was likely to be due, at least in part, to the anti-oxidant properties of the molecule.

OP 20

Cardiovascular disease in type 2 diabetes

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Diabetes specific changes in the macrovasculature: gene array, structural and functional studies

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Backgrounds and Aims: The underlying explanation for the increase in atherosclerosis in diabetes remains to be fully delineated. Thus the aim of this study was to identify novel mechanisms underlying the pathology of potentially diabetes specific atherosclerosis.
Materials and Methods: ApoE-/- mice were randomised to control (C), streptozotocin diabetes (D) or fed a high fat diet (H) at 6 weeks of age so that D and H mice had the same level of aortic plaque at the conclusion of the 20 week study as assessed by *en face* quantitation. The cellular changes in plaques were assessed using immunohistochemistry. Pooled aortic RNA from each of the three groups was used for gene profiling experiments employing the Affymetrix microarray platform. Vessel reactivity was assessed using organ bath studies. Full cumulative dose-response curves to noradrenaline (NA), acetylcholine (Ach), sodium nitroprusside (SNP), endothelin-1 (ET-1) and urotensin II (UII) were constructed. The response to a single dose of angiotensin II (AII, 20 μM) was also obtained. All responses to constrictors were normalised for vessel diameter.
Results: D mice had increased plasma glucose and glycated haemoglobin levels and decreased plasma insulin levels compared to C and H. High fat feeding had no effect on insulin levels. Both D and H had an approximate 10-fold increase in aortic plaque area compared to C (C,1.1 ± 0.4; D,9.5 ± 2.1, H,12.5 ± 1.3%; $p<0.001$). Despite no significant difference between D and H plaque area, microarray analysis of pooled aortic RNA revealed differential expression of over 100 genes, including those involved in fatty acid and iron metabolism. In addition a range of proinflammatory molecules including serum amlyoid A1, a known ligand to the receptor for advanced glycation endproducts, was upregulated in D but not H mice. Immunohistochemistry revealed that D mice had increased macrophage accumulation in the vessel wall compared to C, an effect not seen in H mice. (C, 15 ± 2; D,23 ± 1; H,13 ± 2% $p<0.001$). A similar effect was observed with respect to α-smooth muscle actin staining (C,14 ± 1; D,32 ± 2; H18 ± 3% $p<0.001$). Compared to C, D mice demonstrated a dampened response to Ach (EC50 C,–7.2 ± 0.1; D,–6.9 ± 0.1 $p<0.05$) and an increased response to NA (EC50 C, –8.3 ± 0.1; D,–8.6 ± 0.1 $p<0.05$). D mice also had an increased response to AII compared to H ($p<0.05$). Taken together these data suggest a possible reduction in nitric oxide availability in D mice. In addition D mice had a reduced maximal response to UII compared to C and H ($p<0.05$). H mice were less responsive to NA than C and D mice ($p<0.05$) although response to NA was increased by approximately 60% when NOLA was added (Emax NA,0.54 ± 1.0; NA+NOLA,0.88 ± 0.21 $p<0.05$). Maximal response to ET-1was also reduced in H compared to C mice ($p<0.005$), together suggesting there may be an increase in nitric oxide availability in the vessels of H mice.
Conclusions: Interestingly this study demonstrates specific differences in 1) vessel structure 2) vessel reactivity and 3) gene regulation in aortae of diabetic mice compared to high fat fed mice. These findings extend our understanding of diabetes specific changes in the vasculature relevant to atherosclerosis and could lead to new therapeutic targets in this setting.

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Angiotensin II modulates the glucose-dependent migration to platelet-derived growth factor-BB in human aortic vascular smooth muscle cells by signalling through the statin-sensitive MAPK/ERK pathway

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Background and Aims: In diabetes glucose modifies cell signalling events associated with the development of hypertension and atherosclerosis. Platelet-Derived Growth Factor (PDGF) potentiates vascular smooth muscle cell (VSMC) migration into atheromatous plaques, while angiotensin II is elevated in hypertension. PDGF-BB acts on the tyrosine kinase receptor PDGF-beta, while angiotensin II signals via G-protein coupled receptors.
Aim: To assess the interactions of high glucose (25 mM: HG) and angiotensin II on PDGF-beta receptor expression and chemotaxis of primary aortic VSMC.
Materials and Methods: The levels of receptors and ERK1/2 (key second messenger protein in the MAPK pathway) were assessed using immunoprecipitation and Western blot. ERK activation was assessed using an ERK activity assay. Chemotaxis, recorded using the Dunn chamber, was analysed by cyclical statistics.
Results: Chemotaxis of VSMC occurred to supraphysiological levels of PDGF-BB (0.7–1.1 nM) in 5 mM glucose (normal glucose: NG) or at lower levels (0.2–0.7 nM PDGF-BB) in HG ($p<0.01$). At physiological levels of PDGF-BB (< 0.5 nM), HG caused an increase in the concentration of PDGF-beta receptor in cultured VSMC ($p<0.0001$, vs. NG). At higher levels of PDGF-BB (>0.5 nM) in HG receptor concentration decreased; there is therefore a biphasic response in receptor concentration as PDGF-BB level increases in HG. The receptor response in HG was accompanied by biphasic changes in ERK phosphorylation and activation ($p<0.0001$ vs. NG). Angiotensin II only caused VSMC chemotaxis in HG, at concentrations between 10–20 nM ($p<0.01$). In HG, angiotensin II (10 nM) caused a significant increase in ERK activation and PDGF-beta receptor ($p<0.05$ vs. NG for both). ERK phosphorylation, PDGF-beta receptor upregulation, and chemotaxis to both ligands was blocked by inhibitors of the MAPK/ERK pathway (lovastatin or PD98059: both 10 micromolar). The biphasic response to the ligands in HG was driven through the phosphatase PP2A since its inhibitor, Endothall (90 nM), restored chemotaxis to cells in 1.8 nM PDGF-BB or 30 nM angiotensin II and increased both ERK phosphorylation and PDGF-beta receptor level.
The combined effect of angiotensin II and PDGF was investigated. In HG when 10 nM angiotensin II was combined with 0.4 nM PDGF-BB (independently, each caused chemotaxis) the PDGF-beta receptor level significantly decreased ($p<0.05$) and chemotaxis was not detected, probably through phosphatase activation.
Conclusion: The biphasic response in the PDGF-beta receptor and chemotaxis to PDGF-BB in HG can be altered in the presence of angiotensin II which also signals through the statin-sensitive MAPK/ERK pathway.

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Expression and function of receptors for insulin-like growth factor-I and insulin in human coronary artery smooth muscle cells

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Background and Aims: Hyperinsulinemia, insulin resistance as well as low insulin-like growth factor-I (IGF-I) have been implicated in the pathogenesis of cardiovascular disease. Little is known about direct effects of IGF-I and insulin on human coronary artery smooth muscle cells (HCASMC). Our aim was to characterize the expression and function of insulin-like growth factor-I receptor (IGF-IR) and insulin receptor (IR) in HCASMC.
Materials and Methods: Cultured HCASMC were used. mRNA expression was measured by quantitative real-time RT-PCR analysis. Receptor proteins, phosphorylation of β-subunits and the presence of Hybrid IR/IGF-IR were analyzed by immunoprecipitation and Western blot. DNA synthesis and glucose metabolism was assessed using ^{3}H-thymidine incorporation and D-[U^{14}-C]-glucose accumulation, respectively.
Results: The mRNA expression of IGF-IR was approximately 10 fold higher than that of IR and 80 fold than that of IGF-I in HCASMC. By immunoprecipitation and Western Blot analysis both IGF-IR and IR could be demonstrated. Phosphorylation of the IGF-IR β-subunit was obtained by IGF-I 10^{-10}–10^{-8} mol/l and insulin 10^{-8} mol/l. Insulin and IGF-I 10^{-10}–10^{-8} mol/l phosphorylated the IR β-subunit. When immunoprecipitated with monoclonal α-subunit IR or IGF-IR antibodies we found bands in slightly different positions suggesting the presence of Hybrid IR/IGF-IR. IGF-I 10^{-9}–10^{-8} mol/l significantly stimulated ^{3}H-thymidine incorporation and at a concentration of 10^{-9}–10^{-7} mol/l also D-[U^{14}-C]-glucose accumulation in HCASMC. Insulin 10^{-9}–10^{-7} mol/l had no effect on DNA synthesis, but increased glucose accumulation at 10^{-7} mol/l.
Conclusion: Our study provides experimental evidence for a role of IGF-IR and possibly hybrid IR/IGF-IR in HCASMC.

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Polymorphisms in the sceletal muscle glycogen synthase and apolipoprotein E genes on chromosome 19q13 predict cardiovascular mortality in a gender specific fashion

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Background and Aims: Cardiovascular disease is the leading cause of death among patients with type 2 diabetes (T2D). Genes coding for the skeletal muscle glycogen synthase (*GYS1*) and apolipoprotein E (*ApoE*) are located within 4 Mb on chromosome 19. We considered the possibility of allelic association between earlier reported risk alleles in *GYS1* and *ApoE* and investigated whether they could predict cardiovascular mortality in T2D family members. It has been shown that carriers of the rare allele of the *GYS1* XbaI polymorphism cannot increase their glycogen synthase protein level in response to exercise. With this in mind we hypothesized that the level of physical activity could modulate the genetic prediction of cardiovascular mortality.
Material and methods: A total of 4660 subjects aged >35 years (2145 males, 2515 females, age 58.2 ± 13.8 years, 34% with T2D and 41% with the metabolic syndrome) participating in the Botnia study in Finland were genotyped for *GYS1* (intron 14, XbaI C/T) and *ApoE* (-219G/T, ApoEε2/ε3/ε4) polymorphisms using allelic discrimination or single base pair extension on the ABI3100 or ABI7900. Mortality was assessed after a median follow up of 7.9 years from a national death-certificate registry using Cox regression analyses with robust variance estimate, adjusted for sex and family correlations.
Results: During the follow up time, 749 individuals had died (16.1%) and of them 409 due to cardiovascular disease (8.8%). Gender specific univariate analyses showed that among males the *GYS1* XbaI CT/TT (hazard ratio 1.8 [1.2–2.6], p=0.0016) but not the ApoE risk-genotype combination (ε3/ε4 or ε4/ε4 together with –219 TT, p=0.14) was a significant predictor of cardiovascular mortality. In contrast, amongst females the *ApoE* risk-genotype combination (2.3 [1.6–3.2], p<0.0001) but not the *GYS1* XbaI CT/TT (p=0.18) was a significant predictor. Multivariate analyses using stepwise forward inclusion revealed that low physical activity, T2D, earlier cardiovascular events, smoking and the *GYS1* XbaI CT/TT genotypes were significant risk factors for cardiovascular mortality in males (p<0.0001), whilst low physical activity, high fasting plasma glucose level, earlier cardiovascular events, hypertension, BMI and the *ApoE* risk-genotype combination were significant predictors in females (p<0.0001). While a low level of physical activity by itself was a strong predictor of CVD mortality (2.9 [2.1–4.0] and 2.6 [1.9–3.6] in males and females respectively, p<0.0001), the *GYS1* XbaI CT/TT predicted cardiovascular mortality particularly in men with normal physical activity level (2.2 [1.4–3.7], p=0.0014) while the *ApoE* risk-genotype combination predicted CVD mortality in females reporting low physical activity level (2.4 [1.4–4.1], p=0.00080).
Conclusions: In the Botnia study, polymorphisms in *ApoE* and *GYS1* predict cardiovascular mortality in a gender specific fashion and the risk is dependent on the level of physical activity.

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The effect of pioglitazone on intima media thickness is uncoupled from its glucose lowering effects in patients with type 2 diabetes mellitus

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Background and Aims: Increased Carotid Intima Media Thickness (CIMT) is a well established predictor of cardiovascular and cerebrovascular disease in patients with type 2 diabetes. PPARγ activation by pioglitazone was shown to improve several cardiovascular risk factors and to decrease CIMT in diabetic and in non-diabetic patients. This study was performed to evaluate the effect of pioglitazone on CIMT in comparison to glimepiride under consideration of the glucose lowering effects of both drugs.
Materials and Methods: One-hundred-seventy-three type 2 diabetic patients were randomly assigned to pioglitazone 45 mg (55 men, 34 women; age (Mean±SD) 62.2 ± 8.4 years; duration of diabetes 89.0 ± 94.8 months; HbA1c 7.5 ± 0.9%) or glimepiride 1–6 mg treatment (52 men, 32 women; age (Mean±SD) 63.0 ± 7.4 years; duration of diabetes 82.5 ± 77.5 months; HbA1c 7.4 ± 0.9%). At baseline and following 6 months of study intervention, HbA1c, insulin resistance ($HOMA_{IR}$-Score) and CIMT (B-mode ultrasound) were measured. CIMT was evaluated in a separate reading centre by a single investigator blinded to the treatment assignment of the patients. In addition, patients were stratified according to their metabolic responsiveness into high responders (HR: change in HbA1c > 0.72) and low responders (LR: change in HbA1c ≤ 0.72).
Results: Despite similar significant improvements in metabolic control (HbA1c: pioglitazone –0.8 ± 0.9% vs. glimepiride –0.6 ± 0.8%; n.s. between the groups), additional improvements in HOMA-Score (–2.21 ± 3.40; p<0.0001), and CIMT (–0.033 ± 0.052 mm; p<0.0001) could be observed for pioglitazone but not for glimepiride treatment. Reduction in CIMT was found to correlate with an improvement in $HOMA_{IR}$-Score (r=0.29; p=0.0003), but not with HbA1c reduction (r=0.03; p=0.68). The percentage of changes in HbA1c, $HOMA_{IR}$, and CIMT in HR and LR in both treatment groups is given in the table below.
Conclusion: In our study, PPARγ stimulation by pioglitazone was found to improve glucose control and CIMT in patients with diabetes mellitus type 2. This improvement in CIMT was independent from the glucose lowering effects of the drug. These findings might have important treatment implications.
Percent changes after 6 months of treatment($ p<0.05 vs. baseline; * p<0.05 between groups)

	Pioglitazone HR (n=38)	Pioglitazone LR (n=43)	Glimepiride HR (n=32)	Glimepiride LR (n=49)
HbA1c	–18.0 +/– 7.1$	–2.7 +/– 6.3*	–16.2 +/– 4.7$	–1.9 +/–6.9*
HOMA Score	–31.1 +/– 48.6$	–17.1 +/– 38.0$	2.6 +/– 57.4	14.5 +/– 57.8
CIMT	–5.0 +/– 6.0$	5.9 +/– 6.0$	–2.0 +/– 6.2	–0.6 +/– 6.4

Support: TAKEDA

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Effect of atorvastatin on stroke in patients with type 2 diabetes: factors predicting the risk of stroke in the Collaborative Atorvastatin Diabetes Study (CARDS)

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Background and Aims. Patients with type 2 diabetes are at increased risk of stroke, however, tools for predicting risk of primary stroke are limited. In UKPDS, duration of diabetes, age, gender, smoking, systolic BP, TC:HDL ratio and atrial fibrillation were significant predictive factors. The aim of this retrospective analysis is to summarize the effect of atorvastatin treatment on stroke and factors predictive of stroke in CARDS, a trial of 2838 patients with type 2 diabetes, with no history of CHD or macrovascular disease, who were randomized to treatment with atorvastatin 10 mg or placebo and followed for a median of 3.9 yr.
Materials and Methods. The first stroke among those patients that experienced such an event was classified as fatal or nonfatal. Treatment groups were compared on time to stroke by a Cox regression model. Relationships between individual baseline factors and time to stroke were assessed in separate Cox regression models with adjustment for treatment. Factors significant at the 0.05 level were subsequently entered into a single multivariable model.
Results. Treatment with atorvastatin 10 mg was associated with a 48% RRR for stroke (21 atv vs 39 pbo; p=0.016). Eight of the strokes were fatal (1 atv, 7 pbo), and 52 nonfatal (20 atv, 32 pbo). Adjusted for treatment allocation, older subjects were at higher risk of experiencing a stroke (mean for patients who developed a stroke vs. those who did not: 66 vs. 62 yr; Hazard Ratio (HR) for 10 y increment = 2.19, p<0.001), as were subjects with longer duration of diabetes (10 vs. 8 yr; HR for 10 y increment = 1.49, p=0.020) and higher SBP (148 vs. 144 mmHg; HR for 10 mmHg increment = 1.17, p=0.044). Baseline HbA1c was marginally significant (8.1 vs. 7.8%; HR for 1% increment = 1.17, p=0.072) but patients with HbA1c > 10% were at significantly higher risk (HR=2.35, p=0.019). Women were at lower risk of experiencing a stroke (percent for patients who developed a stroke vs. those who did not: 13 vs. 32%; HR=0.32, p=0.003) while subjects with ACR > 2.5 mg/mmol/L (43 vs. 24%; HR=2.40, p<0.001) or a history of retinopathy (43 vs. 30%; HR=1.72, p=0.038) were at higher risk. There were no statistically significant relationships between BMI, baseline lipids, current smoking or presence of atrial fibrillation and the risk of stroke. Interaction tests indicated relationships between each factor and the risk of stroke did not

depend on randomized treatment. When treatment and the significant baseline covariates were entered together in a single multivariable model, treatment was associated with a 50% RRR (p=0.011), and age, gender, ACR > 2.5 mg/mmol/L and HbA1c > 10% remained significant (all p<0.05).
Conclusions. In CARDS, along with age and gender, diabetes-specific variables ACR and HbA1c were important predictors of stroke independent of treatment. Despite the lack of association between baseline LDL-C and stroke, there was a reduction in the risk of stroke associated with atorvastatin treatment. These observations underscore the need for diabetes-specific risk engines for estimating risk of stroke and for intensive management of modifiable risk factors in patients with type 2 diabetes.
Support: Diabetes UK, UK Department of Health, Pfizer Inc

OP 21
Continuous glucose monitoring

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Evaluation of a new non-invasive blood glucose monitoring device for critically ill and surgical patients
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Background and Aims: Critically ill patients frequently experience abnormalities in carbohydrates metabolism and a severe insulin resistance state. Hyperglycemia is a negative predictor of outcome in these patients, as high blood glucose (BG) values are associated with an increased risk of complications. Recently, based on prospective study, the target of glucose levels in intensive care units (ICU) was set between 80 and 110 mg/dl. These target levels reduce patient mortality by as much as 45%, setting the stage for the need of continuous glucose monitoring in critical care setting. Currently, BG is monitored by measuring capillary blood glucose from fingertips or from venous/arterial line blood samples. It is evident that such monitoring cannot fulfill the need for continuous glucose monitoring in order to safely implement tight glucose control protocols. The purpose of this study is to evaluate the feasibility of the NBM-100 device for non-invasive continuous BG monitoring in critically ill patients.
Materials and Methods: The NBM-100 uses a finger-based sensor shaped as a ring, located at the finger's root. It uses Red/Near-Infrared spectroscopy to detect and analyze BG levels and hemoglobin concentrations. The NBM-100 utilizes an enhanced optical signal resulting from a temporary over-systolic occlusion, produced by a finger-based pneumatic cuff. The resulting changes in the optical absorption and scattering create the sensitivity needed for measuring the BG concentrations.
A study was conducted on 15 patients (5F, 10M, ages 27–84), 7 in the operation room and post-surgery recovery unit of Kaplan Medical Center, and 6 in the intensive care unit of Sheba Medical Center. In order to evaluate the performance of the NBM-100 system for continuous monitoring in these settings, the NBM-100 probe was placed on the patients' thumbs, where it performed continuous BG measurements for 2–12 hours, after receiving an informed consent from patient or relatives. The NBM-100 applied a measurement once every 10–15 minutes. Results obtained from the NBM-100 device were compared to arterial blood samples taken through an arterial line every 30–60 minutes and analyzed with a POC blood gas machine (Nova Biomedical).
Results: A retrospective analysis based on a universal parameter selection with personal adjustment of the coefficients was performed on the NBM-100 readings for all 15 patient cases to calculate the standard error values for the total of 432 data points. Glucose range was 57–256 mg/dl. The resulting mean relative absolute error (RAE) was 8.3%, the mean absolute error (MAE) was 11.5 mg/dl. A Clark error grid analysis showed 98.6% of the measurements fall within zones A and B.
Conclusion: This preliminary study indicates the potential use of the NBM-100 as a truly non-invasive sensor for prompt and continual BG evaluation and improving patients care. Its application in a clinical setting remains to be studied further with a larger study group, longer sessions and a wider BG range.

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Continuous glucose monitoring in a medical intensive care unit
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Background: Critical illness induces counterregulatory hormones and administration of catecholamines, corticoids, and nutritional support may lead to hyperglycaemia. It is unknown whether maintaining euglycaemia (80–110 mg/dl) using intensive insulin therapy is feasible in a medical intensive care unit (MICU) and whether it reduces morbidity or mortality. Continuous glucose monitoring (CGM) might improve adjustment of insulin therapy.
Aims: To assess whether insulin regimens (SC vs IV), based on discontinuous glucose monitoring, can achieve euglycaemia in a MICU.
Patients & Methods: 50 adult patients (M/F: 31/19, age 62 ± 16 y, non-diabetic/type 2 DM/type 1 DM: 30/16/4, IV vs SC insulin: 22/28; APACHE-II score: 22, range 5–42, SOFA score: 8, range 1–16) were recruited in a single-

center, prospective, observational study. 48 h-CGM was initiated within 3 days after admission at ICU, using a subcutaneous glucose sensor (GlucoDay®; Menarini Diagnostics) and compared with arterial blood glucose samples.
Results: During 48 h-CGM, target glycaemia (80–110 mg/dl) was reached in only 22 ± 18% of the time. Glycaemia was >140 mg/dl in 39 ± 27% and <60 mg/dl in 5 ± 10% of the time. The mean insulin dose/day was 71 units (range 0–393). Patients on SC insulin spent more time at glycaemia >110 mg/dl (71 ± 24% vs 55 ± 22% on IV insulin,p=0.016), but similar time at glycaemia <60 mg/dl. Septic shock patients (n=17) had higher insulin needs (133 ± 120 vs 39 ± 54 units/day,p<0.0001, and spent less time at glycaemia >110 mg/dl (55 ± 24 vs 69 ± 24%, p=0.05). Diabetic patients spent more time at hyperglycaemia (%time >140 mg/dl: 51 ± 27 vs 30 ± 24%, p=0.005). Patients with a mean glycaemia>140 mg/dl had a higher BMI (31 ± 9 vs 24 ± 5,p<0.0001) than those with glycaemia ≤140 mg/dl, but APACHE-II and SOFA scores were similar. Presence of diabetes, insulin regimen and dose did not influence mortality. Glycaemia correlated with leucocyte count (r=0.39, p=0.005), haemoglobin (r=0.39, p=0.005), total protein (r=0.32, p=0.022) and lactate concentration (r=0.29, p=0.044), but not with renal and liver parameters. CGM detected peaks (e. g. when starting TPN) and dales of glycaemia much earlier than discontinous monitoring. There were no adverse events with the use of the GlucoDay®.
Conclusions: The GlucoDay® system was well tolerated and constitutes a good method to monitor glucose profiles in MICU patients. Continuous monitoring of glucose levels is mandatory for optimal titration of insulin therapy in the ICU, as target glycaemia between 80 and 110 mg/dl was only reached in 22% of the time. IV insulin therapy is better than a SC regimen to obtain target glycaemia.

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Trans-cutaneous fluorescence lifetime based continuous glucose reading for long term interrogation
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Background and aims: Several attempts have been made to develop continuous glucose monitors that could work for more than three days *in vivo* without the need for frequent re-calibrations. A lot of work has focused on skin penetrating electrochemical based glucose sensors. However, due to regulatory demands they are limited to three days use. PreciSense is working to develop a biocompatible and biodegradable glucose sensor, which can work for at least 14 days *in vivo* and may be interrogated optically and trans-cutaneously. The sensor is injected by a patient operated disposable device and resorbed by the body after use.
Materials and methods: Two different glucose sensor systems designed for *in vivo* non-invasive trans-cutaneous interrogation were tested in a laboratory set-up. Both systems are based on determining the degree of Förster Resonance Energy Transfer (FRET) in the system by measuring the fluorescence lifetime. The two systems are 1) a proprietary system consisting of a Glucose Receptor of human origin (HGR) and Dextran and 2) the well known ConA/Dextran system. The systems were tested during 14 days periods and delivered glucose readings every 5 minutes. The glucose concentration was changed between four levels.
With the HGR based system a short-term pre-clinical experiment was carried out. A sensor pellet was injected in the dermis of an anaesthetized pig (Danish Landrace, female, 45 kg). 6 hours after insertion of the sensor the glucose concentration was stepped to 30 mM by IV glucose infusion and held constant for 2 hours followed by a hyperinsulinemic euglycemic clamp (1.0 mU insulin/kg/min with a variable glucose infusion). The arterial glucose of the pig during the run-in period was reached and upheld for additional 2 hours. The pre-clinical experiment lasted 13 hours.
Results: In the *in vitro* set-up both systems exhibit excellent stability and no demand for re-calibration during the two-week test period. For the HGR based system the 14 days *in vitro* test showed an average repeatability of the glucose measurements as good as 0.2 mM (Standard Deviation, SD) at a level of 3 mM glucose and 3% (Coefficient of Variation, CV) at a level of 30 mM glucose based on a single calibration on the first day of the test. For the ConA based system the *in vitro* test showed an average repeatability of the glucose measurements as good as 0.05 mM (SD) at a level of 2.5 mM glucose and 2% (CV) at a level of 25 mM glucose based on a single calibration on the first day of the test.
In the pre-clinical *in vivo* experiment total reversibility of the sensor was observed. After a step to 30 mM arterial glucose concentration a lag between rise in arterial glucose concentration and the onset of sensor response of 5 minutes was observed. The sensor response reached 90% of the steady state level after 58 minutes. After the step from 30 mM to 7 mM the lag between decrease in arterial glucose concentration and the onset of sensor response was 5 minutes.
The average repeatability of the glucose concentration estimate obtained with the sensor was 13% (CV) at the 7 mM level and at the 30 mM level.
Conclusion: The *in vitro* results prove long-term stability of the sensor, while the pre-clinical experiment confirms the functionality of the sensor in an *in vivo* environment. In conclusion, the results point towards the possibility of developing a stable minimally invasive commercial glucose reader for at least 2 weeks continuous use.

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Guardian® RT Continuous Glucose Monitoring System with real time glucose values and alarms functions: a new tool for improving glucose control in patients with type 1 diabetes mellitus?
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Background and Aims: The Guardian® RT (Medtronic MiniMed) Continuous Glucose Monitoring System is a subcutaneously self-inserted electroenzymatic glucose sensor with continuous telemetered display of real time glucose values, and automatic alerts for preset hypo- and hyperglycaemic levels. The clinical utility of the device was investigated ambulatory during ordinary life conditions.
Materials and Methods: 12 adults (aged 23–60 yrs) and 12 children (8–18 yrs) with T1DM on intensive insulin therapy (17 CSII, 7 MDI) were randomly recruited, and were asked to wear the device over a 10-days period. The sensors were replaced by the patients themselves every 3 days. Average 24 h glucose levels, frequency of hypo- and hyperglycaemic alarms, patients' own use of the real time glucose values and alerts, and treatment changes were assessed.
Results: 22 patients used the sensor system continuously for 9–14 days. At the start, the median low and high alarm thresholds were set at 3.5 mmol/l (range 3.0–4.5 mmol/l) and at 11.1 mmol/l (9.0–13.0 mmol/l), respectively. 11 patients self-adjusted the alert settings during the study. On average, 2.4 hyper and 1.4 hypo alarms/daytime and 0.8 hyper and 0.6 hypo alarms/night were encountered. 21 patients actively used the real time glucose readings and the alarms to modify their insulin doses, diet, and/or life style. Comparing the first and last day of the study period, the number of glucose excursions above 11.1 mmol/l decreased on average by 38% (p<0.05). Moreover, in patients with a mean 24 h glucose level ≥6.7 mmol/l on day 1 (n=20), a significant improvement in glycaemic control was registered at the end of the study phase (reduction of mean 24 h glucose concentration from 9.6 ± 2.6 mmol/l to 8.1 ± 1.8 mmol/l, p<0.05). Six mild adverse events (four related to sensor insertion) were reported in four patients.
Conclusion: The Guardian® RT provides continuous and clinically useful information about the actual glycaemic control, facilitating active self-adjustments of diabetes management by the patients. The results also suggest that the device may help to improve daily glycaemia. This hypothesis is presently being investigated in 162 T1DM patients (81 children and 81 adults) with inadequate glycaemic control (HbA_{1c} 9.6 ± 1.2%) in a randomised, controlled (standard self-monitoring of capillary blood glucose), multi-center trial for a three month period, evaluating metabolic outcomes, with the projected completion during the spring of 2005.
Support: Medtronic MiniMed

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Comparison of a needle type and a microdialysis continuous glucose sensor in type 1 diabetes patients
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Background and aims: Our aims were to examine needle type CGMSgold® (MiniMed) and microdialysis based GlucoDay® (Menarini) sensor accuracy during the night and rapid glucose excursions, to verify hypothesized nocturnal hypoglycaemic drift for the needle type and delay for the microdialysis based sensor. Also, we re-examined the 'push-pull phenomenon', i.e. the hypothesis that interstitial glucose is delayed upon a blood glucose rise,

while interstitial glucose precedes a fall in BG. We used a novel, physiological way to induce rapid glucose changes and analysed the large number of interdependent values using statistical methods, new in the field of continuous glucose sensoring.
Materials and methods: Overnight venous blood was sampled twice/hr in 13 Type 1 diabetes patients. 30 Min after breakfast, rapid acting insulin was given sc. Sampling once/min started 45 min after breakfast and 75 min after insulin injection for 30 min, aiming at peak and nadir glucose.
Mean Absolute Differences (MAD's) were assessed and a Clark Error Grid was constructed. For each patient the two sensor curves were first separately modelled by natural splines using linear regression. Hereafter, combined curve fitting was applied, assuming that the sensor curves have the same shape as the BG curve, allowing for horizontal and vertical shift away from the BG curve. Vertical (horizontal) shift is the vertical (horizontal) distance between sensor and BG curve indicating drift resp. delay. The push-pull phenomenon should be recognizable by the morning peaks of the separately fitted sensor curves being narrower than the peak of the BG curve. Therefore, width of the morning peak of the three methods was assessed and compared per patient.
Results: MAD was 16.1% (735 paired samples) for CGMSgold and 13.8% (1156) for GlucoDay (P=0.28, paired-samples t-test). In the Error Grid analysis, needle type resp. microdialysis method exhibited 72.4 and 76.0% of the paired values in zone A, 24.4 and 22.3% in B, 0 and 0.1% in C, 4.1 and 1.5% in zone D. Mean vertical shift was –0.04 resp. –0.02 mmol/l for the needle type and the microdialysis based sensor. Mean (SD) horizontal shift was 7.1 min (± 5.5) for the microdialysis sensor (P<0.001, one-sample t-test). No horizontal shift was found for the needle type sensor (–2.2 min± 6.6, P=0.28, minus sign indicates a shift to the left). Correction for 7 min delay improved GlucoDay MAD to 12.6% (P=0.07 vs. CGMSgold, P=0.08 vs. uncorrected GlucoDay, paired samples t-test). Mean peak width of the needle type (110.0 min± 20.5) and microdialysis sensor (104.6 min± 21.7) paradoxically tended to exceed mean BG peak width (100.0 min± 24.0; P=0.052 neelde type, P=0.11 microdialysis, 2-sided paired Wilcoxon test).
Conclusions: The microdialysis based approach seems potentially more accurate than the needle type approach. The current version of the needle type sensor did not show a nocturnal hypoglycaemic drift, as the previous version did. Inherent delay for the microdialysis system has been quantified by the horizontal shift. Although this delay can be corrected for retrospectively, there are implications for prospective use of the device as hypoglycaemic alarm. Physiological delay between blood and interstitial glucose has not been confirmed by the needle type sensor according to the horizontal shift. Assessment of peak widths did not support the push-pull phenomenon.

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Clinical utility of the continuous glucose monitoring system in intensified insulin treated patients

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Background and Aims: Clinical use of the continuous glucose monitoring system (CGMS) provides significantly more information on glucose patterns than self-monitoring of blood glucose (SMBG). CGMS may reveal unrecognised hyper- and hypoglycemia previously unseen using SMBG. The aim of this study was to analyze continuous glucose profiles and uncover correctable factors hidden from detection with conventional SMBG.
Materials and Methods: Performance data and demographic information were obtained from total of 47 type 1 poorly controlled diabetic patients (mean age±SD 23,47 ± 11,33 years, average duration of diabetes 7,83 ± 7,34 years, mean HbA1c=9,56 ± 1,48%, BMI=21,98 ± 3,67; 5 on continuous subcutaneous insulin infusion, 42 on multiple daily injections). Patients were monitored using the CGMS for 3 or more days during normal activity, in conjunction with SMBG tests conducted at least 4 times per day. Duration, frequency and cause of hyper- and hypoglycemic excursions were analyzed. Results were presented as means±SD and percentage of time period spent hypo- or hyperglycemic. We compared HbAlc at baseline and 3 months after CGMS-based therapy changes. Statistical analysis included correlation, means absolute difference and paired Student's t test.
Results: 51 390 glucose values obtained from continuous glucose monitoring with a mean duration of 97,67 ± 28,44 h were evaluated by comparing to 949 capillary blood glucose (BG) values (Δr=0,86 ± 0,09; mean absolute difference =16,37 ± 5,31%). CGMS detected total of 645 hypoglycemic episodes (<3,5 mmol/l at least 10 min). From 166 nights only 2,2% were hypoglycemic, but 5 episodes of asymptomatic hypoglycemia were prolonged (>280 min), 63% was during 1am–4am period, 35 from 38 hypoglycemic episodes were undetected by SMBG (p<0,0001). 35% of day-time hypoglycemia was during 10am–1pm period, only 1 of 3 episodes was symptomatic and detected by SMBG. The CGMS uncovered lowered BG post-exercise, continued fall for 1,5 h post-exercise and an average lower BG following day, in 3 cases (ice hockey, ice skating) with nocturnal hypoglycemia. Subjects showed 38,8% prevalence of hyperglycemia (Δ 9,3 h/patient/day), total of 9761 hyperglycemic episodes (>7,8 mmol/l at least 10 min), mostly attributed to diet fault or insufficient lag time. 75% of posthypoglycemic hyperglycemia >10 mmol/l was caused by overeating, 20% was attributed to rebound hyperglycemia. Dawn Phenomenon was detected in 4 subjects. Stress hyperglycemia (work-stress, car-driving, pit bull terriers' attack) tended to come down without additional insulin. HbA1c significantly decreased from 9,56 ± 1,48% at baseline to 8,86 ± 1,38% within 3 months after changes in management based on the CGMS results (p=0,021).
Conclusions: In conclusion, the CGMS uncovered correctable factors hidden from detection with SMBG and completed picture of patients' glycemic responses to sleep, work, exercise, food intake and insulin dose. CGMS-determined therapy adjustments showed improved glycemic control. The study provides additional support for the clinical usefulness of the CGMS in the intensified insulin management of the diabetic patients.

OP 22

Beta cell dysfunction and death

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Is there a role for locally produced inflammatory mediators in glucotoxicity to human pancreatic islets?

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Background and Aims: Different degrees of β-cell failure and apoptosis are probably present in type 1 and type 2 diabetes mellitus (T1D and T2D). Inflammatory mediators may cause β-cell apoptosis in T1D, and it has been suggested that nutrient-induced β-cell apoptosis in T2D shares a final common pathway with T1D, involving interleukin-1β (IL-1β) production by the β-cells, nuclear factor-κB (NF-κB) activation and death via Fas-FasL. The aim of this study was to test whether glucotoxicity in human islets is related to IL-1β production and consequent NF-κB activation.
Materials and Methods: Human islets were isolated from five cadaveric organ donors in Uppsala. The islets were cultured free-floating for 48 h and 7 days in RPMI 1640 medium with 10% fetal calf serum at 5.6, 11 or 28 mM glucose. For comparative purposes, islets were exposed to IL-1β alone (50 U/ml, at 5.6 mM glucose, n=5) or in combination with interferon-γ (IFN-γ, 1000 U/ml, n=3). β-Cell purity of the islets was 46 ± 5% (mean±SEM). mRNA expression levels were assessed by real time RT-PCR in a blinded fashion and corrected for the house keeping gene β-actin. IL-1β, IL-1 receptor antagonist (IL-1ra) and insulin were measured by ELISA.
Results: Culture of the human islets for 48 h at 11 and 28 mM glucose induced a 4–5-fold increase in medium insulin as compared to 5.6 mM glucose ($p<0.05$). IL-1β mRNA expression did not vary at different glucose concentrations (94 ± 36 at 5.6 mM, 55 ± 12 at 11 mM and 107 ± 48 at 28 mM glucose after 48 h, n=5), and neither did IL-1ra (85 ± 23, 122 ± 15 and 93 ± 19, respectively). In keeping with the mRNA data, IL-1β release to the medium was unchanged by glucose (8.6 ± 4.9 pg/50 islets x 48 h at 5.6 mM, 3.3 ± 2.1 at 11 mM and 4.1 ± 2.5 at 28 mM glucose, n=5), as was IL-1ra release (98 ± 37 pg/50 islets x 48 h at 5.6 mM, 91 ± 38 at 11 mM and 102 ± 55 at 28 mM glucose). In comparison, stimulated human monocytes released over 50-fold more IL-1β (n=3). Expression of the NF-κB-dependent genes Iκ-Bα and MCP-1 was induced in human islets by IL-1β but not by high glucose (Iκ-Bα: 68 ± 9 at 5.6 mM, 69 ± 12 at 11 mM, 69 ± 6 at 28 mM glucose and 309 ± 90 in the presence of IL-1β, $p<0.01$ for IL-1β vs 5.6 mM glucose; MCP-1: 74 ± 23 at 5.6 mM, 42 ± 7 at 11 mM, 62 ± 22 at 28 mM glucose and 303 ± 92 in the presence of IL-1β, $p<0.01$ for IL-1β vs 5.6 mM glucose). IL-1β + IFN-γ also induced by 2–3-fold Iκ-Bα and MCP-1 expression. Inducible NO synthase was not induced by glucose or IL-1β alone, but its expression increased more than 50-fold in the presence of IL-1β + IFN-γ. There was also no glucose-induced Fas expression (127 ± 39 at 5.6 mM, 83 ± 14 at 11 mM and 142 ± 24 at 28 mM glucose after 48 h, n=5), the proposed NF-κB-dependent mechanism by which glucose causes β-cell death. Similar observations were made after exposure of the human islets to different glucose concentrations for 7 days.
Conclusion: High glucose does not induce IL-1β production and NF-κB activation in human islets *in vitro*, suggesting that locally produced inflammatory mediators do not play a role in glucotoxicity. Our findings argue against a unifying hypothesis for the mechanisms of β-cell death in T1D and T2D and suggest that different approaches will be required to prevent β-cell death in both diseases.
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Chronic exposure to high glucose concentration results in reduced tyrosine phosphorylation of IRS-2 and impairs the PI3-KINASE/AKT insulin signaling pathway in mouse pancreatic B-cells

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Background and Aims: Type 2 diabetes is characterized by insulin resistance of peripheral tissues, pancreatic beta-cell dysfunction and apoptosis. Recent investigations have provided functional and genetic evidence for an autocrine role of insulin on the signaling cascade in β-cell growth, survival and function. The main signaling pathway used by the insulin receptor originates with tyrosine phosphorylation of IRS-1/IRS-2 (insulin receptor substrate) leading to sequential activation of phosphatidylinositol 3-kinase (PI3-kinase) and its downstream effector, the Ser/Thr kinase PKB/Akt. Akt plays a central role in promoting protein synthesis and cell survival. In the light of increasing evidence that insulin signaling plays an important role in the functioning of β-cells, we investigated whether chronic exposure of mouse pancreatic β-cells (βTC-6 cells, an insulin-secreting beta-cell line) to high glucose concentration results in alterations in early steps of insulin signaling pathway.
Materials and Methods: Murine insulin secreting β-cells (βTC-6 cells, ATCC Cat. No. CRL-11506) were continuously grown in DMEM culture medium containing 1, 5 or 25 mM glucose, 15% FCS, 4 mM glutamine and the effects of glucose on early steps of insulin signaling were examined by western blotting analysis using specific antibodies.
Results: We demonstrated that chronic exposure of βTC-6 cells to high glucose concentration (5 or 25 mM glucose) resulted in a 40–50% reduction of insulin-stimulated total tyrosine phosphorylation of IRS-2 compared with control cells grown in the presence of 1 mM glucose. In contrast, insulin-stimulated tyrosine phosphorylation of IRS-1 was not affected. Expression of IRS-1 and IRS-2 did not differ between βTC-6 cells exposed to high glucose concentration and control cells. Glucose-induced down regulation of insulin-stimulated IRS-2 tyrosine phosphorylation also resulted in a 50% reduction of IRS-2-associated PI-3 kinase levels. In addition, insulin-stimulated Akt activation was also impaired as indicated by a 75% reduction in Ser473Akt phosphorylation. No difference in Akt content was observed between cells exposed to 5 or 25 mM glucose and cells exposed to 1 mM glucose. The PI3-kinase/Akt signaling pathway plays a central role in promoting cell survival by modulating numerous downstream survival and cell death factors including pro-apoptotic and anti-apoptotic proteins of the Bcl-2 family. Therefore the observed glucose-induced impaired activation of Akt might be accompanied by alterations in the expression and/or activity of the pro-apoptotic proteins Bad and Bax and/or the anti-apoptotic members Bcl-XL and Bcl-2 of the Bcl-2 family. This hypothesis is currently under investigation.
Conclusion: In conclusion, our results demonstrate that chronic exposure of pancreatic β-cells to high glucose concentration impairs the IRS-2/PI3-kinase/Akt insulin signaling pathway and suggest that defects in early steps of insulin signaling may play a role in determining β-cell dysfunction and apoptosis observed in type 2 diabetes.
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Opposite effects of glucose on the unfolded protein response and the integrated stress response in rat islets

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Background and Aims: Accumulation of misfolded proteins in the endoplasmic reticulum (ER) activates the ER stress sensors Ire1, ATF6 and PERK. The specific Unfolded Protein Response (UPR), characterized by increased expression of chaperones (BiP, GRP94) and components of the ER-associated degradation pathway (EDEM), results from Ire1-mediated XBP1 mRNA splicing and ATF6 activation. The unspecific Integrated Stress Response (ISR), characterized by translational pause and stimulation of ATF4 target gene expression (GADD153, GADD34…), results from eIF2α phosphorylation by PERK or others kinases. Both disruption and excessive activation of ER stress can induce β-cell apoptosis and diabetes. It has also been proposed that excessive stimulation of proinsulin synthesis by hyperglycemia could overwhelm the ER protein folding capacity and trigger ER stress in β-cells. We therefore tested whether glucose triggers an ER stress in rat islets.
Materials and Methods: Rat islets were precultured for 1 week in RPMI containing 10 mM glucose (G10) and 5 g/L BSA. They were then cultured 18 h in the presence of G2, G5, G10 or G30 and various test substances. After culture, islet gene expression was measured by RT-PCR and western blot.
Results: As expected, thapsigargin (TG), an inhibitor of SERCA pumps that triggers ER stress by depleting ER Ca^{2+} stores, rapidly and dose-dependently increased the spliced/total XBP1 mRNA ratio in G10 (from 0.27 ± 0.01 (mean±SE) to 0.63 ± 0.03 and 0.70 ± 0.03 after respectively 2 and 4 h treatment with 1 μM TG, n=3, $P<0.01$) without affecting total XBP1 nor TBP mRNA levels. TG (8–18 h) also significantly increased GADD153, GADD34, BiP, GRP94 and EDEM mRNA levels (respectively 6.1 ± 2.0, 9.5 ± 1.0, 4.3 ± 0.2, 3.1 ± 0.1 and 3.4 ± 0.1 fold increase vs G10, n=3, $P<0.05$). In comparison, glucose exerted distinct effects on these various ER stress markers. After 18 h culture, the spliced/total XBP1 mRNA ratio increased from 0.12 ± 0.01 and 0.10 ± 0.01 in G2 and G5 (NS) to 0.19 ± 0.01 in G10 and

0.32 ± 0.02 in G30 (n=7, P<0.01). In contrast, glucose stimulation decreased GADD153 mRNA and protein levels (mRNA levels in G2, G5 and G30 relative to G10: 11.5 ± 2.0 (P<0.01), 6.8 ± 0.8 (P<0.01) and 1.4 ± 0.1 (NS), n=7). Under these conditions, EDEM and GRP94 mRNA levels were respectively increased ~1.4 and ~1.9 fold by G30 vs G2-G10, whereas BiP mRNA levels were ~2 fold higher in both G2 and G30 vs G5-G10. Interestingly, the opposite effects of glucose on XBP1 mRNA splicing and GADD153 expression were rapid, reaching a maximum within 2 h of stimulation with G30 after 18 h culture in G5. These glucose effects were not affected by stimulation of insulin secretion with tolbutamide, nor by its inhibition with diazoxide or clonidine. In contrast, inhibition of protein synthesis by 10 µM cycloheximide completely prevented the increase in XBP1 mRNA splicing and GRP94, BiP and EDEM mRNA levels from G5 to G30 while reducing total XBP1 mRNA levels by 50% and increasing GADD153 mRNA levels at all glucose concentrations.
Conclusion: In contrast with TG that activates a full ER stress response in cultured rat islets, glucose rapidly activates the specific UPR in a protein synthesis dependent manner while inhibiting the unspecific ISR. The rapidity of these effects suggest their possible role in nutrient-induced maintenance of the β-cell phenotype.

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Transcriptional regulation of the endoplasmic reticulum (ER) stress-induced gene CHOP in pancreatic beta-cells
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Background and Aims: Type 1 cytokines, such as interleukin-1β (IL-1β) and interferon-γ (IFN-γ), are potential mediators of beta-cell death in type 1 diabetes mellitus. Under *in vitro* conditions, IL-1β + IFN-γ induces NO production and death by apoptosis of pancreatic islet cells. We have recently shown that cytokine-induced NO production by the beta-cells triggers the ER stress response and increases the expression of the pro-apoptotic transcription factor CHOP. Of note, iNOS blockers prevent both cytokine-induced CHOP expression and beta-cell death (our own data), and islets from CHOP KO mice are protected against NO-induced cell death (Oyadomary et al. 2001). Against this background, we presently studied the molecular regulation of CHOP expression in insulin producing cells. For this purpose, we used wild type and deletion mutants of the CHOP hamster promoter linked to the luciferase reporter gene.
Materials and Methods: INS-1E cells were transfected with different luciferase reporter plasmids containing wild type (WT, –782bp) or deletion mutants [MuB (–437bp), MuC (–229bp), MuE (–63bp), MuF (–782bp + deletion of the endoplasmic reticulum responsive element (ERSE)] of the hamster CHOP promoter and the internal control pRL-CMV. Sixteen to 24 h after transfection the cells were exposed to: 1. medium alone for 15–36 h; 2. IL-1β (10 U/ml) + IFN-γ (100 U/ml) for 36 h; 3. cyclopiazonic acid (CPA, 25 µM; a SERCA blocker and ER stress inducer) for 15 h and 4. carrier solvent of CPA (DMSO, 0.04%) for 15 h. Luciferase activities were assayed with the dual-luciferase reporter assays system and the test values were corrected for the value of the internal control plasmid.
Results: Transient transfections were performed in INS-1E cells to test the ability of IL-1β + IFN-γ or CPA to induce CHOP promoter activity. There was a basal CHOP activity with the –782 bp wild type (WT) promoter that was increased by respectively 2 ± 0.1 and 3.2 ± 0.5 fold after cytokine or CPA exposure (p<0.05 vs. non treated cells n=7). Deletion of nucleotides –782 to –437 bp had no effects on the basal, cytokine or CPA induced CHOP promoter activity. On the other hand, deletion of nucleotides –437 to –229 bp decreased basal promoter activity [71.8 ± 11.2 relative luciferase unit (RLU) WT promoter vs. 37.1 ± 7.9 RLU MuC, n=7 p< 0.05] and CPA induction (2.0 ± 0.3 fold, p<0.05 vs. WT, n=7), and completely abolished cytokine induction. This region contains a C/EBP-ATF composite site which has been implicated in CHOP regulation by ER stress via ATF4. Deletion of the endoplasmic reticulum responsive element (ERSE) significantly decreased CPA induction of the CHOP promoter (2.3 ± 0.3 fold, p<0.05 vs. WT, n=7) but did not affect its induction by cytokines, indicating differential regulation of CHOP by cytokines and the SERCA blocker.
Conclusions: The present study shows that cytokines and CPA regulate CHOP expression at the transcriptional level in insulin producing cells. IL-1β + IFN-γ induces the CHOP promoter via activation of a C/EBP/ATF composite site, while the SERCA blocker CPA regulates CHOP expression via both C/EBP/ATF and ERSE sites.
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Rescue of glucolipotoxic INS-1E cells by adenoviral overexpression of carnitine-palmitoyl transferase 1 involves expression changes of multiple beta-cell proteins
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Background and aims: The mechanisms by which prolonged exposure to elevated levels of glucose and fatty acids negatively affect the pancreatic β-cell (glucolipotoxicity) remain unclear. Although the combined elevated nutrient levels promote accumulation of fatty acid and triglycerides and generation of ceramide, the role of these processes in deterioration of β-cell function are less clear. Given the proposed role of carnitine-palmitoyl transferase 1 (CPT1) in carbohydrate and fatty acid fuel partitioning via malonyl-CoA induced down-regulation, the fatty acid shuttling mitochondrial protein has been investigated. The role of CPT1 in β-cell glucolipotoxicity has been challenged e. g. by studies where CPT1 was blocked by etomoxir. The aim of the present study was to further investigate mechanisms of β-cell glucolipotoxicity. To this end, CPT1 was overexpressed or not in INS-1E cells, which were cultured in the presence of elevated levels of glucose and oleate. Whereas INS-1E cells not overexpressing CPT1 showed signs of glucolipotoxicity by not responding to elevated glucose levels with enhanced insulin secretion, INS-1E cells overexpressing CPT1 demonstrated glucose-stimulated insulin secretion (GSIS). To identify new proteins involved in glucolipotoxicity, protein profiles of INS-1E cells overexpressing CPT1 or not were generated and compared.
Material and methods: INS-1E cells were seeded in 24-well plates and cultured at 11 mM glucose and 10% FCS for 48 hours, after which they were infected or not by an adenoviral vector containing the CMV-promoter (Ad-CMV) or the liver type CPT1 under the control of the Tet-On promoter (Ad-Tet-On-CPT1). Subsequent culture, which lasted for another 48 hours, was performed in the presence of 20 mM glucose and 0.5 mM oleate. In Ad-Tet-On-CPT1 infected INS-1E cells, 1 µM doxycyclin was added to the culture medium. After culture, INS-1E cells were used for determination of GSIS, CPT1 levels and global protein profiles. Insulin released at 3 or 11 mM glucose was determined by ELISA. CPT1-levels were determined by Western blotting and chemiluminescence detection. Global protein profiles were generated by protein arrays analysed by surface enhanced surface laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS).
Results: INS-1E cells, which had been infected or not with Ad-CMV, demonstrated no increase in insulin release, when the glucose concentration was elevated from 3 to 11 mM. In contrast, 2-fold increase in GSIS was observed in Ad-Tet-On-CPT1 infected INS-1E cells. The latter cells had an almost 8-fold increase in CPT1-levels compared to CPT1-levels of INS-1E cells infected or not with Ad-CMV. Global protein profiles revealed several differentially displayed peaks, when profiles of non-infected INS-1E cells were compared with profiles of INS-1E cells infected with Ad-Tet-On-CPT1.
Conclusions: Alleviation of glucolipotoxicity, as measured by restoration of GSIS, was achieved by enhanced expression of CPT1. CPT1 overexpression caused changes in expression of multiple β-cell proteins. The identities of these differentially displayed proteins may provide new insights for the molecular mechanisms of glucolipotoxicity.

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Reduced β-cell mass in a transgenic mouse MODY3 model is caused by excessive apoptosis in pancreatic islets
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Background and Aims: Maturity-onset diabetes of the young (MODY) is a monogenic disease representing 2–5% of all cases of type 2 diabetes. The most common form is MODY3, which is caused by mutations in the transcription factor hepatocyte nuclear factor-1α (HNF-1α) gene. HNF-1α regulates the expression of several genes i.e. glucose transporter-2, L-type pyruvate kinase, and insulin. However, whether HNF-1α affects β-cell mass is not known. We therefore examined β-cell mass and its balancing mechanisms, apoptosis and proliferation, in a transgenic mouse model with β-cell-targeted expression of dominant-negative HNF-1α (DNHNF-1α). This mouse model has previously been shown to express high non-fasting blood glucose levels, impaired glucose elimination and reduced islet insulin content.
Materials and Methods: In DNHNF-1α and wild type (wt) C57BL/6J mice β-cell mass was studied in whole pancreas using insulin staining and stan-

dardized sectioning sampling. Islet apoptosis was studied by caspase 3/7 activity and islet proliferation by Western blot analyses of protein kinase B α (Akt1) and pancreatic duodenal homeobox-1 (Pdx-1) expression. We also assessed maximal insulin secretory capacity in these mice by performing a maximal insulin secretion test (MIST), in which a mixture of arginine/glucagon-like peptide-1/glucose (0.25, $1.04*10^{-5}$, and 1 g/kg respectively) was administered iv and the area under the insulin curve during the first 50 minutes after injection was determined. Finally, we also evaluated glucose oxidation in isolated islets.
Results: The *in vivo* MIST revealed a severely reduced maximal insulin secretion capacity in DNHNF-1α mice when compared to wt animals (174 ± 20 vs. 27 ± 3.6 nM*50 min, p<0.001). β-cell mass was reduced by 18% in DNHNF-1α mice compared to wt animals (0.91 ± 0.05 vs. 1.12 ± 0.06 mg, p=0.025). This was seen in association with a 23% increased islet caspase 3/7 activity (p<0.01) and an unaltered islet expression of Akt1 and Pdx-1 protein. Glucose oxidation was reduced by 35% in islets from DNHNF-1α mice at 16.7 mM glucose concentration (p=0.032).
Conclusion: In association with a marked reduction of the maximal insulin secretory capacity, the β-cell mass was reduced in DNHNF-1α mice due to increased apoptosis and no change in proliferation rate. Also glucose metabolism, as judged from glucose oxidation data, was reduced in DNHNF-1α islets. This suggests that a combination of reduced β-cell mass and functional perturbations underlie the islet defects in MODY3.

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Diabetes in childhood

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Benefits of rosiglitazone in children with type 2 diabetes mellitus
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Background and Aims: The occurrence of type 2 diabetes mellitus (T2DM) in children is rapidly increasing, but treatment options are limited. The effect of rosiglitazone (RSG) monotherapy was evaluated in a 24-week, double-blind, randomised, metformin-controlled (MET), parallel group study in 195 T2DM children previously treated with either diet and exercise (drug-naive) or oral monotherapy (prior therapy).
Materials and Methods: After a 4-week placebo run-in/wash-out period, children were randomised to RSG 2 mg bid or MET 500 mg bid. At week 8 doses were up-titrated to RSG 4 mg bid or MET 1000 mg bid, as needed to attain fasting plasma glucose < 7.0 mmol/l.
Results: Demographic and baseline characteristics were comparable between groups: M:F = 1:2; mean age 14 years (range 8–17); mean weight 90 ± 30 kg; mean BMI 33.6 ± 9.2 kg/m^2. HbA_{1c} was well-matched at screening (RSG = 8.17%; MET = 8.06%), but was poorly matched at randomisation/baseline (RSG = 7.88%; MET = 8.17%). Distributional assumptions underlying protocol-defined analyses were not met; however, non-parametric analyses revealed statistically significant median reductions in HbA_{1c} from baseline (RSG = –0.25%, $P = 0.0276$; MET = –0.55%, $P < 0.0001$) and screening (RSG = –0.5%, $P = 0.0110$; MET = –0.5%, $P = 0.0037$) to week 24 in both groups. Differences between groups were not statistically significant. The difference between HbA_{1c} in RSG and MET groups during the run-in period was impacted by prior oral monotherapy. Drug-naive subgroups from both groups showed consistent reductions in HbA_{1c} and achieved similar mean HbA_{1c} values by week 24 (RSG = 7.49%, n = 55; MET = 7.22%, n = 50). RSG was generally safe and well-tolerated in children. Mild peripheral oedema was seen in only one child receiving RSG. At week 24, mean weight gain with RSG was 3 kg and none with metformin. RSG pharmacokinetics in children were consistent with those previously described in adults.
Conclusion: RSG is a safe and effective glucose-lowering therapy in children with T2DM and may represent another potential treatment option.

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High prevalence of elevated albumin excretion in youth with type 2 diabetes: the SEARCH for Diabetes in Youth Study
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Background and Aims: Diabetic nephropathy is an important cause of end stage renal disease in adults. There is a paucity of data on the burden of diabetic nephropathy in children, especially according to diabetes (DM) type. The prevalence of elevated albumin excretion, a marker of diabetic nephropathy, was assessed according to DM type in SEARCH for Diabetes in Youth, a U.S. six-center, multi-ethnic study of youth less than 20 years of age with physician-diagnosed DM.
Materials and Methods: A spot urine sample was obtained from 2633 youths with DM (age 13.7 ± 4.5 years, 50% male, DM duration 5.1 ± 3.9 years) and albumin and creatinine were measured. Elevated albumin excretion was defined as a urine albumin to creatinine Ratio (ACR) ≥30 µg/mg. DM type was defined based on SEARCH-measured diabetes autoantibodies (DA) and fasting C peptide (FCP): T1a = positive DA and low or preserved insulin secretion (FCP < 3.7 ng/ml); T1 = insulinopenia (FCP < 0.8 ng/ml) without DA; T2 = high insulin secretion (FCP ≥ 3.7 ng/ml) without DA; Hybrid = features of both T1a (positive DA) and T2 (FCP ≥ 3.7 ng/ml); Undetermined Type= individuals with preserved insulin secretion (FCP 0.8–3.7 ng/ml) and no DA.

Results: The prevalence of elevated ACR was 10.9%, 9.5% in youth with DM duration <5 years and 12.7% in those with DM duration ≥5 years. The prevalence of elevated ACR (Table) was significantly higher among youth with T2, hybrid and undetermined DM type, compared with youth with T1a DM. In multiple logistic regression analyses, adjusted for age, gender, race/ethnicity, DM duration, hemoglobin A1c and systolic blood pressure, youth with non-type 1 DM (T2, hybrid and undetermined types combined) were 3.1 (95% CI 2.1–4.6) times as likely to have an elevated ACR as those with T1a/T1 DM. Further adjustment for differences in body mass index did not change this finding. Risk for elevated ACR was similar among non-Hispanic white and minority youth, when adjusted for the above covariates.
Conclusion: Youth with T2 DM are at higher risk for elevated albumin excretion than those with T1 DM, independent of age, gender, DM duration, race/ethnicity, glycemic control and blood pressure. Given the suspected increase in incidence and earlier age at onset of both T1 and T2 DM, the burden of diabetic renal disease may increase as these youths mature. Targeted screening and treatment programs may be necessary to prevent this outcome.
Prevalence of elevated ACR by DM type

	T1a	T1	T2	Hybrid	Undetermined
Total N	1872	508	60	27	166
Elevated ACR N (%)	176 (9.5%)	48 (9.4%)	17 (28.3%)	7 (25.9%)	38 (22.9%)
P-value	Reference	1.0	<0.0001	0.01	<0.0001

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The PedPump survey: a low percentage of basal insulin and more than seven daily boluses are an option for better glycaemic control in 1086 children on CSII from 17 countries

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Background and Aims: The number of paediatric patients treated with CSII is increasing rapidly in many countries. The aim of this survey was to document current paediatric practices using insulin pump in paediatric care in children with type 1 diabetes, and to assess therapeutic strategies and outcomes of CSII under real life conditions.
Materials and Methods: This multicentric survey was conducted in 30 centres from 17 countries. The studied population included 1086 paediatric patients (0–18 y); mean age: 11.9 ± 4.2, diabetes duration: 5.9 ± 3.6 (0.2–18 y), CSII duration: 2.0 ± 1.4 (0.2–14.5 y), HbA1c: 8.0 ± 1.3%, BMI: 19.7 ± 3.4 kg/m^2. Over a three month period, clinical data, centralised HbA1c in DCCT-standard laboratory with HPLC method, and pump memory readout of patients treated with Medtronic MiniMed pumps were recorded using an electronic data collection system.
Results: Glycaemic control was better in preschool (n=145, 7.6 ± 1.0%) and prepubertal (6 to 11 y, n=322, 7.7 ± 1.1%) than in adolescent patients (12–18 y, n=604, 8.3 ± 1.4%). Intensity of insulin therapy with CSII was reflected in a high average number of boluses per day (7 ± 4). Children taking more than 7 boluses per day had a significantly better HbA1c (7.6 ± 1.0 vs 8.4 ± 1.4%, p<0.001) than those with less boluses. The average contribution of basal insulin in total daily dose was below 50% in all groups: 34.1% in preschool, 42.2% in prepubertal and 46.4% in adolescent groups. Children with less than 44% of their total daily insulin as basal (group median) had also significantly better HbA1c than those with higher basal rate percentages (7.7 ± 1.1 vs 8.4 ± 1.3%, p < 0.0001). The rate of severe hypoglycaemia was 7.09 per 100 patient years and was not associated with the number of boluses.
Conclusion: This large paediatric survey of patients on CSII demonstrates the benefits of insulin pump therapy with low rates of hypoglycaemia. The use of frequent daily boluses and a low rate of basal insulin in pediatric patients treated with pumps is an adequate therapeutic option for a better glycaemic control.

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Standardized documentation for external quality control reveals significant changes in pediatric diabetes care during the last 10 years

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Background and Aims: External comparison (benchmarking) is established for continuous quality improvement. It has been adapted to medicine during the last 20 years. Meanwhile many medical fields have integrated benchmarking into their quality management approach. However, few initiatives managed to work continuously for many years with a large, homogeneous group of caregivers.
Material and Methods: Supported by the German Secretary of Health a computer software (DPV) for the standardized documentation of structure, process and outcome of paediatric diabetes care was developed at the University of Ulm. Data anonymously collected by this software are externally compared among participating institutions, separately for paediatric and adult diabetes centres. Following a data validation step,benchmarking is offered twice yearly. Feedback to centres includes histograms of relevant quality indicators, bi- and multivariate analyses, local discussion circles and a yearly meeting of the whole group.
Results: In 2004, 143 German and 3 Austrian paediatric centres participated in the DPV quality comparison compared to 55 centres in 1995. Average centre size was stable with 98 paediatric patients (age < 18 years) per centre in this period. In 2004, intensified insulin regimen (4 and more daily injections) was used by 63.9% of patients (1995: 23.8%), insulin pump therapy by additional 9.8% (1995: 0.6%). In parallel, blood glucose monitoring (BG) increased dramatically from 34% of patients obtaining 4 or more BG values per day in 1995 to 78% in 2004. In contrast, 15.5% of patients checked their urine once or more per day in 1995, compared to only 4.9% in 2004. Duration of re-hospitalisation after diabetes onset declined significantly from 7.2 inpatient days per patient and year in 1995 to 4.8 days in 2004. But, outpatient patient contacts stayed at 4.3 visits to the diabetes centre per year. In contrast, outcome indicators like metabolic control (median DCCT-adjusted HbA1c: 7.9%), rate of severe hypoglycaemia (seizure/coma/convulsion: 4.5 events/100 pat.-years, "help required": 23.5) and rate of hospitalization for DKA (3.4 events/100 pat.-years) remained stable.
Conclusion: Our data demonstrate the feasibility of standardized external comparisons for quality control over a 10-years-period. The joint data analysis objectively monitors changes in the process and outcome of diabetes care. Benchmarking reveals characteristics of individual centres and provides the basis for continuous quality improvement. Reduction in inpatient care reflects - at least in part - current economic influences on diabetes care.
Acknowledgment: The DPV system for standardized diabetes documentation in paediatric and adult diabetes care is supported by grants from: German Diabetes Association, German Diabetes Foundation and NovoNordisk Germany.
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The evaluation of the selected aspects of the emotional state of the children and adolescents with type 1 diabetes in dependence on metabolic control and insulin therapy method

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Background and Aims: The study evaluating the insulin therapy methods concentrate on chronic complications prevention (DCCT, EDIC – type 1 diabetes, UKPDS – type 2 diabetes). A very important goal is also proper physical, psychological and emotional development. It is very difficult to achieve in chronic disease like diabetes. You can expect higher anxiety level, which can influence on normal emotional functioning and can disturb proper development. The aim of study was the evaluation of the anxiety level and coping strategies of the children and adolescents with type 1 diabetes in relation to metabolic control and used insulin therapy method.

Materials and Methods: 34 patients in the age range of 7,4 to 19,3 years (mean 15,0) suffering from type 1 diabetes took part in the study. They were divided into 3 groups in dependence on insulin therapy method (conventional, intensive, with personal insulin pump) and on metabolic control level. The evaluation of the emotional functioning was performed on the base of the psychological methods (Coping Inventory for Stressful Situations and State-Trait Anxiety Inventory).
Results: We have found higher anxiety level as trait than state in all diabetic patients (p=0,005) independently on insulin therapy and metabolic control. We have observed the high use of the task-oriented coping in the group of patients treated with personal insulin pump and IIT (p<0,01 and p<0,001 respectively). Patients treated with KIT demonstrated more emotion-focused coping responses than CSII group (p<0,02). The high anxiety level as state (NS) and higher use of avoidance-oriented coping (limit of significance) was associated with the poorest metabolic control (HbA1c>8,5%). The anxiety-state level was significantly higher in patients with HbA1c range 7–8,5% than in the group with the best metabolic control (p<0,05). We mentioned a tendency to attenuation of task-focused coping responses according to the metabolic control deterioration.
Conclusion: 1. High level of anxiety as trait occurs in all diabetic patients and is independent on the insulin therapy method and the metabolic control. 2. Patients treated with personal insulin pump and with intensive insulin therapy demonstrate the predominant use of the task-oriented coping style.

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Prevalence of type 1 diabetes in a Bangladeshi population based on the presence of GAD and IA2 antibody
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Background and Aims: Classical type 1 diabetes mellitus is thought to be relatively rare in Bangladesh on clinical ground. However, a substantial number of diabetic populations have onset of disease in early childhood and adolescence. They are mostly nonketotic but do not show features of classical type 2 diabetes. In an attempt to characterize these young diabetic patients we estimated anti-GAD and IA-2ic antibodies in a cohort of young diabetic patients and healthy Controls.
Materials and Methods: Study subjects comprised of young diabetic subjects (n=314) with diabetes diagnosed before 30 years; fibrocalculous pancreatic diabetes (n=41); and nondiabetic healthy controls (n=280) with negative family history of diabetes up to second generation. Anti-GAD and IA-2ic antibodies were determined by radioimmunoprecipitation method and C-peptide was estimated by ELISA.
Results: Young diabetic and FCPD subjects had no difference in their mean (±SD) age (19.4 ± 6.3 and 19.9 ± 6.0 respectively). C-peptide (mean±SD, nmol/l) levels of the three groups were 0.43 ± 0.19 in the controls, 0.29 ± 0.26 in young diabetics (p<0.0001 vs Controls) and 0.17 ± 0.16 in FCPD (p<0.0001 vs Controls). Prevalence of anti-GAD and IA-2ic antibodies among the controls were respectively 3.2% and 0.4% in controls; 22.6% (71/314) and 11.8% (37/314) in young diabetics and 19.5% (8/41) in FCPD. Twenty five individuals (7.96% of all diabetics) were positive for both anti-GAD and IA-2ic antibody. Among the FCPD subjects 6 (14.6%) were positive for both the humoral markers. The antibody positive diabetic subjects were of significantly younger age compared to negative cases [16.5 ± 6.4 and 19.7 ± 6.1 years (p<0.001)]. The positive cases had significantly higher fasting glucose [16.8 ± 5.8 and 14.8 ± 6.7, mM (p=0.024), and low C-peptide levels [0.19 ± 0.16 and 0.33 ± 0.28, nM (p<0.001)] compared to negative cases. The antibody positive FCPD cases also showed similar trends.
Conclusion: Positivity for GAD and IA-2ic antibody in the Bangladeshi population is almost similar to those found in European population. Substantial number of positivity for anti GAD and IA-2ic antibodies among young diabetic and FCPD subjects suggest: i) prevalence of type 1 diabetes may be more than that is usually thought on clinical experience; and ii) antibody positive cases have low but residual C-peptide and this may mask their clinical presentation of ketoacidosis.

OP 24
Insulin action

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Intrinsic differences in insulin signaling responses in human visceral and subcutaneous adipocytes
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Background and Aims: Visceral fat accumulation is central to the development of metabolic abnormalities and increased cardiovascular risk in humans. Whether adipocytes from this fat depot feature specific and intrinsic biochemical properties has not been elucidated. The aim of this study was to investigate potential differences of insulin signaling reactions in human subcutaneous and visceral pre-adipocytes differentiated *in vitro* into adipocytes.
Materials and Methods: Paired abdominal subcutaneous (SC) and omental (O) fat biopsies were obtained from non-obese subjects with normal glucose tolerance. Fat stromal cells were separated from mature adipocytes and endothelial cells, cultured to confluence, and differentiated *in vitro* into mature adipocytes with insulin, dexamethasone, T3, and rosiglitazone. Expression and phosphorylation levels of signaling intermediates, i. e. Akt and Erk-1/2, that are key for regulation of adipocyte metabolism and gene expression were analyzed under basal conditions, and following insulin stimulation (10 nM) for 6 min and 30 min.
Results: Akt protein content was similar in SC and O adipocytes (p=0.88). Basal Akt phosphorylation on Ser473 and Thr308 was also similar in the two cell populations. Insulin induced a significant increase in Akt phosphorylation on Ser473 (p<0.05 vs. basal), which was evident 6 min following insulin stimulation and remained sustained up to 30 min in both SC and O cells. Akt phosphorylation on Thr308 was persistently stimulated by insulin up to 30 min in SC adipocytes (p<0.05 vs. basal at all time points), but more transiently induced in O adipocytes with peak phosphorylation at 6 min (p<0.05 vs. 30 min). Protein content of Erk-1 and Erk-2 was 32% and 82% higher in O than SC adipocytes, respectively (p<0.05), and basal phosphorylation of Erk-2 was also moderately increased in O adipocytes (p<0.05 vs. SC adipocytes). Noteworthy, in O adipocytes, insulin stimulation induced rapid increases in Erk-1 and Erk-2 phosphorylation at 6 min (p<0.05 vs. basal), which returned to basal levels at 30 min. By contrast, stimulation of Erk phosphorylation by insulin was modest and non-significant in SC adipocytes.
Conclusion: Erk proteins display more rapid and intense activation in response to insulin in O compared to SC adipocytes. A more rapid response of Akt phosphorylation on Thr308 to insulin is also observed in O compared to SC adipocytes, whereas Akt phosphorylation on Ser473 shows similar activation profiles. Thus, even when differentiated *in vitro* from precursor stromal cells, visceral adipocytes display dynamic signaling responses that may explain the intrinsic metabolic flexibility of visceral fat.
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In skeletal muscle cells advanced glycation end products inhibit insulin action by inducing the formation of a multi-molecular complex which includes RAGE, PKCalpha and IRS1
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Background and Aims: Insulin resistance in diabetes is genetically determined, but its occurrance is also affected by factors secondary to diabetes itself. These secondary factors further impair insulin action in the diabetic individual. For instance, chronic hyperglycemia per se induces insulin resistance. One mechanism through which hyperglycemia exacerbates insulin resistance is the increased production of Advanced Glycation End products (AGEs). We have previously demonstrated that AGEs impair insulin signalling in L6 skeletal muscle cells, by activating Protein Kinase C (PKC) alpha and preventing IRS1/2 tyrosine phosphorylation, followed by selective inhibition of insulin-stimulated glucose metabolism. However, the molecular mechanism of AGE-induced PKCalpha activation has not been defined yet.

Materials and Methods: We have investigated this issue in cultured L6 skeletal muscle cells.
Results: We investigated whether the receptor for AGEs (RAGE) could directly interact with PKC in response to AGEs. We first analysed the interaction of RAGE with different PKC isoforms. Treatment with AGEs (glycated albumin 0.1 mg/ml) for 24 h selectively induced co-precipitation of RAGE with PKCalpha but not with other PKCs such as PKCbeta, delta and zeta. RAGE-associated PKCalpha activity was increased by about 3-fold upon 24 h incubation of the cells with AGEs ($p<0.01$). Moreover, based on immunoblotting with specific antibodies, RAGE precipitates contained Ser657 phosphorylated PKCalpha, corresponding to the active form of the protein. Interestingly, AGEs incubation for 24 h also stimulated PKCalpha-IRS1co-precipitation. Overlay blotting of RAGE precipitates revealed that recombinant PKCalpha bound to a 175 kDa band, corresponding to IRS1, suggesting that IRS1 may be necessary for RAGE-PKCalpha interaction. Consistent with this hypotesis, blocking IRS1 expression with a specific ribozyme in L6 skeletal muscle cells prevented the co-precipitation of RAGE with PKCalpha and inhibited AGEs-induced PKCalpha activation.
Conclusion: These novel findings indicate that AGEs induce formation of a multi-molecular complex which includes RAGE, PKCalpha and IRS1.This complex may lead to the activation of PKCalpha and to AGE-dependent inhibition of insulin action in L6 skeletal muscle cells.

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In aortic vascular smooth muscle cells of Zucker fa/fa rats insulin signalling via both PI3-K and MAPK pathways is impaired: evidence against the selectivity of insulin resistance in these cells
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Background and aims: It has been described the occurrence of a "selective" impairment of insulin signalling in the insulin-resistant states: i.e., a reduced response to insulin of the Phosphatidylinositol-3-Kinase (PI3-K) pathway in the presence of a normal response of the Mitogen-Activated Protein Kinase (MAPK) pathway. Accordingly, hyperinsulinaemia occurring in vivo to compensate insulin resistance overactivates MAPK cascade, with a consequent increase of MAPK-dependent insulin effects (i.e., actions on cell proliferation, etc). We aimed to evaluate whether insulin-resistance is "selective" also in Vascular Smooth Muscle Cells (VSMC), by measuring insulin ability to activate PI3-K and MAPK pathways in VSMC derived from an animal model of insulin resistance.
Materials and methods: Experiments have been carried out along the "Principles of laboratory animal care" (NIH Publication no. 85-23, revised 1985) in aortic VSMC obtained as primary cultures in our laboratory from lean insulin-sensitive Zucker fa/+ rats and obese insulin-resistant Zucker fa/fa rats. To measure time- and dose-dependent effects of insulin on phosphorylation of Akt (molecule of PI3-K cascade) and of ERK 1-2 (molecules of MAPK cascade), western blots have been carried out in VSMC incubated for 2, 4 and 6 hours with 2 nmol/l human regular insulin. Dose-dependence experiments have been carried out by incubating VSMC with 0.5, 1 and 2 nmol/l insulin for 4 hours. Data derived by densitometric analysis of western blots are expressed as percent of basal values (mean±SEM).
Results: i)insulin-induced Akt phosphorylation was greater in VSMC from Zucker fa/+ rats than from Zucker fa/fa rats ($p=0.0001$): in particular, after 4 hours of incubation, Akt phosphorylation was 130.92 ± 2.68% of basal values ($n=7$, $p=0.0001$) and 106.59 ± 3.12% of basal values ($n=7$, $p=0.056$), respectively; ii)insulin-induced ERK-1 phosphorylation was greater in VSMC from Zucker fa/+ rats than from Zucker fa/fa rats ($p=0.0001$): in particular, after 4 hours of incubation, ERK-1 phosphorylation was 136.62 ± 2.33% of basal values ($n=7$, $p=0.0001$) and 106.95 ± 3.03% of basal values ($n=7$, $p=0.041$), respectively; iii)insulin-induced ERK-2 phosphorylation was greater in VSMC from Zucker fa/+ rats than from Zucker fa/fa rats ($p=0.0001$): in particular, after 4 hours of incubation, ERK-2 phosphorylation was 146.04 ± 3.86% of basal values ($n=7$, $p=0.0001$) and 104.42 ± 2.79% of basal values ($n=7$, ns), respectively. Dose-dependent experiments showed that in VSMC from Zucker fa/+ rats a 4-hour insulin incubation induces phosphorylation of Akt, ERK-1 and ERK-2 already at 0.5 nmol/l ($p=0.046–0.002$): dose-dependence of the insulin effect is deeply reduced in VSMC from Zucker fa/fa rats, where also the highest concentration tested (2 nmol/l) induced very small phosphorylation increases, not higher than that induced in Zucker fa/+ rats by 0.5 nmol/l insulin.
Conclusion: in VSMC from the insulin-resistant Zucker fa/fa rats insulin resistance is not "selective", since it involves both PI3-K and MAPK pathways. These results suggest that insulin signalling via MAPK pathway in VSMC from Zucker fa/fa rats is not enhanced even in hyperinsulinaemic conditions, as the theory of "selective" insulin resistance implies.

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Calreticulin destabilizes GLUT-1 mRNA in vascular endothelial and smooth muscle cells under high glucose conditions
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Background and Aims:Vascular endothelial (VEC) and smooth muscle cells (VSMC) up- or down-regulate their rate of glucose transport under hypoglycemic or hyperglycemic conditions, respectively, by changing the cell content of glucose transporter-1 (GLUT-1) mRNA level and GLUT-1 protein expression and its plasma membrane abundance. Hyperglycemia-induced down-regulation of glucose transport is particularly important as it protects cells against exaggerated intracellular protein glycation and elevated production of glucose-derived free radicals, which contribute to the development of macrovascular disease and atherosclerosis. We have studied the molecular mechanism that downregulates GLUT-1 mRNA levels in bovine aortic endothelial and smooth muscle cells under high glucose conditions, aiming at identifying the *cis*-acting element (CAE) in the 3'-untraslated region (3'-UTR) of GLUT-1 mRNA and its cognate *trans*-acting factor (TAF) that regulates the turnover rate of this mRNA in response to changes in ambient glucose.
Materials and Methods: RNA Mobility Shift Assays (REMSA), UV cross-linking assays and *in vitro* degradation assays of GLUT-1 mRNA ^{32}P-labeled probes were used to study RNA-protein interactions. Mass-spectrometry was employed to identify TAFs. Adenovirus vectors for over-expression of putative TAFs in cells were prepared. Real-Time PCR analysis was used to measure mRNA levels in vascular cells.
Results: A 10-nucleotide sequence ($CAE_{2181–2190}$) in the 3'-UTR of GLUT-1 mRNA exhibited an increased binding capacity of a cytosolic protein from vascular cells that had been maintained at 23 mM in comparison with 2 mM glucose. The specificity of this interaction was confirmed by REMSA and UV cross-linking assays of 3'-UTR ^{32}P-labeled probes, containing or deleted of $CAE_{2181–2190}$, and cytosolic extracts in the absence or presence of competing sense and antisense complimentary unlabeled probes of various regions of the 3'-UTR. Mass-spectrometric analysis identified the interacting protein as bovine calreticulin. Its expression was increased in VEC and VSMC under high glucose conditions. Western blot analysis of UV cross-linked complexes, using anti-calreticulin antibody, identified calreticulin as the binding protein of $CAE_{2181–2190}$. Pure bovine calreticulin that was added to *in vitro* degradation assays of ^{32}P-labeled-GLUT-1 mRNA probes, which contained $CAE_{2181–2190}$, increased their susceptibility to RNase-induced degradation. Adenovirus-driven over-expression of calreticulin reduced GLUT-1 mRNA level in VEC and VSMC that were exposed to normal glucose levels to the same low level observed in control cells, which were maintained at 23 mM glucose.
Conclusion: This study shows that calreticulin, whose expression is increased in vascular cells under high glucose conditions, augments the rate of GLUT-1 mRNA degradation and leads to the down-regulation of glucose transport. Interestingly, calreticulin is a multi-functional protein that also binds calcium in the endoplasmic reticulum, acts as a chaperone and regulates cell adhesion. Thus, calreticulin, whose expression is increased in vascular cells under hyperglycemic conditions, may also alter other critical cellular functions in vascular cells.
Support: The German-Israel Foundation and The Russell Berie Foundation and D Cure-Diabetes Care in Israel

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Chronic AICAR administration and long-term exercise improve insulin sensitivity and changes glucocorticoid receptor gene expression in rat skeletal muscle
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Background and Aims: Insulin resistance plays a significant role in type 2 diabetes and obesity, and is associated with abnormal regulation of glucocorticoid metabolism. Activation of AMP-activated protein kinase by either adenosine analogue 5-aminoimidazole-4-carboxyamide-1-β-D-ribofuranoside (AICAR) or physical activity is known to increase glucose uptake and insulin sensitivity in rat skeletal muscle fibres. The objective of the present study was to investigate, whether an increase in insulin sensitivity after either long-term AICAR administration or exercise was followed by

muscle fibre-type specific changes in glucocorticoid receptor (GCR) and/or β_2-adrenergic receptor (β_2-AR) gene expression
Materials and Methods: Zucker Diabetic Fat (ZDF) (fa/fa) rats, exhibiting insulin resistance at 8 weeks of age and diabetes at 12 weeks of age, were allocated at 5 weeks of age into either a AICAR treated (n = 10), a exercise trained (ET) (n = 11) or a sedentary control group (SZ) (n = 11). Lean Zucker (fa/-) rats (LZ) (n = 11) served as a reference group. Skeletal muscle GCR and β_2-AR mRNA was determined by reverse transcriptase-PCR and HPLC for separation of standard and unknown and quantification
Results: Both AICAR administration and exercise significantly reduced fasting plasma glucose in treatment week 6, 7 and 8 ($P < 0.001$ for AICAR vs. SZ, and $P < 0.002$ for ET vs. SZ), fasting plasma insulin in week 6 and 7 ($P < 0.005$ for AICAR vs. SZ, and $P < 0.011$ for ET vs. SZ) and insulin resistance (HOMA) in week 6 and 7 ($P < 0.005$ for AICAR vs. SZ, and $P < 0.002$ for ET vs. SZ). GCR mRNA levels varied significantly between the four groups in red ($P < 0.001$) and in white ($P = 0.001$) skeletal muscle, with the highest levels in the LZ reference group. The β_2-AR gene expression did not vary significantly between groups. GCR and β_2-AR mRNA levels were positively correlated in red ($r = 0.349$, $P = 0.022$) and white gastrocnemius ($r = 0.402$, $P = 0.008$). GCR mRNA in red muscle was negatively correlated to HOMA in treatment week 6 and 7 ($r = –0.548$, $P = 0.003$ and $r = –0,379$, $P = 0,036$), i. e. high levels of GCR mRNA in insulin sensitive non-diabetic rats.
Conclusion: Chronic AICAR administration and long-term exercise both improve insulin sensi-tivity, and is associated with muscle fibre-type specific changes in GCR mRNA

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D-chiro-inositol(DCI) as an AMP-activated protein kinase (AMPK) activator
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Background and Aims: DCI had a glucose lowering effect according to other previous study. We guess this effect appear through not only insulin signaling pathway but other pathway. AMPK is a cellular energy sensor. Activation of AMPK improve insulin sensitivity. We performed this study to evaluate the glucose-lowering effect of DCI through AMPK activation.
Materials and Methods: The cultured 3T3-L1 preadipocyte treated with insulin, DCI or AICAR. And we checked levels of protein(phospho-PKB, total PKB, phospho-AMPK, and total AMPK) by immunoblot analysis using specific antibodies. Each two groups of 5–6 week age dB/dB mice and C57Bl6 mice were fed with DCI(100 mg/kg) or Ad libitum for two weeks. Four groups of mice defined: control mice fed with Ad libitum(CL), control mice fed with DCI(DD), dB/dB mice fed with Ad libitum(DL), and dB/dB mice fed with DCI(DD). Thereafter we checked levels of leptin and adiponectin, change of body weight, and levels of blood glucose. Also we checked hepatic level of protein(phospho- and total AMPK) extracted from each groups of mice.
Results: DCI phosphorylate PKB protein by dose-dependent manner in 3T3-L1 preadipocyte and the effect of DCI is greater than insulin. Also DCI stimulate phosphorylation of AMPK in 3T3-L1 preadipocyte. The phosphorylation peaked at 30 minute after DCI treatment. The levels of protein of phospho- and total AMPK were decreased in liver of dB/dB mouse than in C57Bl6 control mouse. The levels of AMPK partially recovered in dB/dB mouse after DCI treatment. Body weight and the levels of blood glucose and leptin are higher in D than C groups. Thre are no difference in body weight and levels of blood glucose, leptin, and adiponectin between each L and D group.
Conclusion: DCI phosphorylate PKB in preadipocyte and stimulate phosphorylation of AMPK in preadipocyte and mouse liver

OP 25

Type 1 diabetes: from start to finish – prediction, progression and mortality

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Public health approach to screening for type 1 pre-diabetes in children
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Background and Aims: In the U.S., one in 1:465 children develop type 1 diabetes (T1D) by 10 yrs of age and 1:200 by age 20, following a period of positivity for islet autoantibodies (IA). We have previously shown that brief diabetes education, combined with HbA1c and random BG test every 3–6 mo prevent T1D onset DKA and hospitalization in pre-diabetic children positive for (IA). The goal of this study was to translate observations from prospective cohort studies into a public health approach to early identification of children with pre-diabetes.
Materials and Methods: Diabetes Autoimmunity Study in the Young has screened for high-risk HLA-DR,DQ genotypes 31,760 general population newborns (GPNs) and 1,336 young first-degree relatives (FDRs) of people with T1D. At-risk GPNs (n=1,506) and FDRs (n=913), have been followed for up to 11 yr (median 4.4 yr) for development of IA (IAA, GAD, IA-2) and T1D.
Results: By age 10, 124 children have been positive for IA on two or more occasions and 35 have developed T1D (11 GPNs and 24 FDRs). A state-wide registry of childhood diabetes has identified additional 13 cases of T1D among the 30,254 GPNs who were not followed up. Over 70% (17/24) of the GPNs who developed T1D by age 10 had HLA genotypes found in only 10% of the general population (DR3/4,4/4,4/1,4/8,4/9 with DQB1*0302 or DR3/3). Of the 35 children followed to T1D, 31 have had IA measured every 3–12 mo prior to diagnosis (a total of 303 test points). Of those, 25 (81%), once IA+, remained positive until diagnosis, while 6 had periodically lost IA, following the first positive visit (two were negative at diagnosis). Based on these data, we constructed a series of general population screening models for early detection of pre-diabetes. Testing of the of GPNs with susceptibility HLA genotypes (10% of the population) for IA at 1.5 yr of age would identify 46% of all children in the general population who develop T1D by age 10. Re-testing at 3.5, 6, and 8 yr would improve the sensitivity to, respectively, 62%, 66% and 69%. With IA testing at 1.5 and 3.5 yr, the median interval between first IA+ test and T1D onset would be 35 mo (range 3 mo- 8.2 yr). Addition to the newborn HLA-DR,DQ screening polymorphisms at the insulin gene (-23 HphI) and CTLA-4 gene (promoter C318T) increased positive predictive value of the screening; while addition of the PTPN22/LYP (C1858T) or MIC-A 5.1 polymorphisms did not materially improve predictive value.
Conclusion: In conclusion, newborn screening for HLA-DR,DQ followed by testing of 10% of the general U.S. population for IA at age 1.5 and 3.5 yr could identify the majority of children who develop T1D by age 10. This could significantly reduce morbidity and cost of T1D onset care and offer a window of opportunity for primary prevention.
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Proinsulin level and proinsulin-to-C-peptide ratio complement autoantibody measurement for predicting type 1 diabetes
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Background and Aims: To investigate whether random proinsulin level (PI) and proinsulin-to-C-peptide ratio (PI/C) complement immune and genetic markers for identification of relatives at high risk of type 1 diabetes.
Materials and Methods: Random glycemia, PI, PI/C and *HLA DQ* genotype were determined at initial sampling in 561 nondiabetic first degree relatives positive for diabetes-associated autoantibodies on ≥ 1 occasion and in 561 age- and sex-matched persistently antibody-negative relatives.
Results: During follow-up (median 62 months), 50 antibody-positive relatives (46 at first sampling) developed type 1 diabetes. Initially antibody-positive relatives ($n = 338$) had higher PI/C values than initially ($n = 223$) or persistently ($n = 561$) antibody-negative subjects. PI ($p \leq 0.008$) and PI/C (p

<0.001) were graded according to risk of diabetes being highest in IA-2 antibody (IA-2A)-positive relatives (n = 68) especially when carrying the high risk *HLA DQ2/DQ8* genotype (n = 20) and in prediabetic relatives (n = 46). High PI/C values tended to persist during follow-up and PI/C was inversely correlated with time to diagnosis in prediabetes (p <0.001). In presence of IA-2A (n = 68) PI/C exceeding percentile 66 of antibody-negative relatives conferred a 5-year risk [95% confidence interval] of 67% [50–84%] similar to that conferred by *DQ2/DQ8* heterozygosity but with higher screening sensitivity (83% vs. 47%; p = 0.007). In antibody-positive relatives without IA-2A (n = 270) elevated PI/C was also significantly associated with an increased 5-year risk of diabetes (however <10%). Cox regression analysis confirmed IA-2A and random PI/C as independent predictors of the risk of diabetes (p <0.001).

Conclusion: Random PI and PI/C represent dynamic markers of the beta cell functional state that complement immune markers in identifying relatives at homogeneously high risk of type 1 diabetes eligible for secondary prevention trials.

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Resolution of the IDDM2 type 1 diabetes susceptibility locus by functional genetics in children with newly diagnosed type 1 diabetes. Results from the Hvidøre study group on childhood diabetes

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Background and Aims: The IDDM2 susceptibility locus of type 1 diabetes (T1D) has previously been mapped to a variable number of tandem repeat (VNTR) in the 5' end of the insulin gene (INS). The protective effect of the VNTR class III allele is speculated to be caused by reduced autoimmune reactivity against insulin and a better preserved beta-cell function. In a recent study the IDDM2 locus was remapped to a 2.1 Kb region containing the three common polymorphisms: VNTR, -23*Hph*I and +1140A/C variants. The -23*Hph*I variant is in 99.77% linkage disequilibrium with the VNTR, but because of a likewise close linkage between -23HphI and +1140A/C variants, genetic transmission studies were, however, not able to show which one of these variants is the causal variant associated with T1D. Using functional genetics (genotype-phenotype correlation studies) we therefore investigated the association of the +1140A/C and -23*Hph*I polymorphisms, with residual beta-cell function and insulin autoantibody levels during the first year after diagnosis with T1D.

Materials and Methods: 257 children and adolescents aged < 16 years from 22 centres in 18 countries with newly diagnosed type 1 diabetes were followed for 12 months. A 90-minutes Boost-test (mixed meal) was carried out in each patient at 1, 6, and 12 months after diagnosis to characterise the residual β-cell function (C-peptide, pro-insulin, rise in post-prandial blood glucose). Insulin antibodies were determined simultaneously by a radioligand assay. RFLP-PCR analysis was performed for genotyping of the -23*Hph*I and +1140A/C variants. Comparison of the genotype effect on the endpoints was performed by multiple regression (adjusted for age, sex, BMI, and HLA-risk groups) and non-parametric Kruskal-Wallis test.

Results: The meal-stimulated C-peptide response 12 months after diagnosis significantly associated with the -23*Hph*I polymorphism T/T genotype compared with the A/A and A/T genotypes (319 pmol/l vs 143 pmol/l and 168 pmol/l, p = 0.043), but not with the genotype groups of the +1140A/C variant. Furthermore, the insulin autoantibody levels at 1 month were also significantly lower in the -23*Hph*I polymorphism T/T and A/T genotype groups compared with the A/A genotype group (p <0.03), whereas no effect was seen of the +1140A/C variant (p = 0.16).

Conclusion: The data from the present study indicate that by functional genetics analyses it is possible to distinguish between the pathogenic role of two closely linked variants, -23*Hph*I and +1140A/C, in the IDDM2 locus. These data also imply that the +1140A/C variant is not involved and that the -23*Hph*1 is most likely a marker of the protective effect exerted by the VNTR variant.

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Contribution of the coexistence of HLA-A24, -DQA1*03 and -DR9 to early and acute beta-cell abolishment in type 1 diabetes: a longitudinal study on residual beta-cell function

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Background and Aims: Temporal profiles of beta-cell destruction in type 1 diabetes are heterogeneous, which brings the heterogeneity in the mode of onset and the status of residual beta-cell function in type 1 diabetes. To elucidate the genetic factors contributing this heterogeneity, we assessed residual beta-cell function longitudinally and examined the relationships between temporal profiles of beta-cell abolishment and HLA class I and II alleles in type 1 diabetes.

Materials and Methods: Subjects were 266 type 1 diabetic patients (men/women: 151/114, age at onset 34 ± 14 years, mean ± SD) and 136 normal controls. In 194 type 1 diabetic patients (men/women: 113/81, age at onset: 36 ± 14 years), serum C-peptide levels were assayed longitudinally 4.3 ± 3.0 times during 12.2 ± 10.4 years. Serum C-peptide level less than 0.017 nmol/l at fasting or 0.033 nmol/l at 2-3 hours after meal was regarded as abolishment of beta-cell. In addition to serological typing of HLA-A,-B, -C, and -DR, HLA-A, DRB1, DQA1 and DQB1 alleles were typed by PCR-RFLP methods. According to the mode of onset, type 1 diabetic patients were divided to fulminant type (n=11, characterized by abrupt onset and elevated pancreatic enzymes at onset), acute onset-type (n=139, period before insulin requirement < 1 year) and slowly progressive type (n=99, period before insulin requirement > 1 year).

Results: In HLA-typed type 1 diabetic patients, all but one had HLA-A24 (A*2402) and/or DQA1*03. The cumulative incidence rate (CIR) of beta-cell abolishment was different among the patients with A24 and DQA1*03 (n=97), those with DQA1*03 only (n=58) and those with A24 only (n=19) (4.3, 2.4, 0.9/100 patient-years, respectively, p=0.010). The patients with both A24 and DQA1*03 lost their beta-cell function completely earlier than the remaining patients (CIR: 4.3 vs. 2.0/100 patient-years, p=0.0062). Patients with HLA-DR9 (DRB1*0901) (n=68) showed earlier beta-cell abolishment than those without DR9 (n=115) (CIR: 4.6 vs. 2.3/100 patient-years, p=0.0094). Furthermore, the patients with A24, DQA1*03, and DR9 (n=38) showed strikingly earlier beta-cell abolishment than the remaining patients (n=124) (CIR: 5.7 vs. 2.2/100 patient-years, p=0.0013). The frequency of the patients with A24, DQA1*03, and DR9 was higher in acute onset-type (38.2%, 47/123) than slowly progressive type (13.6%, 12/88, p<0.0001). The frequency of DR2 was higher in fulminant type (45.5%, 5/11) than acute type (9.7%, 13/134, p=0.0071) and slowly progressive type (12.6%, 12/95, p=0.0095), but not different from normal controls (30.9%, 42/136, p=0.33). When excluding fulminant type from the analysis, the patients with DR2 (n=21) less progressed to beta-cell abolishment than those without DR2 (n=152) (CIR: 1.0 vs. 3.1/100 patient-years, p=0.0095).

Conclusion: Coexistence of HLA-A24, DQA1*03 and DR9 contributes to early and acute beta-cell abolishment in type 1 diabetes. DR2 plays a protective role against complete beta-cell destruction.

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Age at onset of diabetes and the development of end-stage renal disease in childhood-onset type 1 diabetes. A nation-wide population-based study

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Background and Aims: To analyse the impact of age at onset on the development of end-stage renal disease (ESRD) due to diabetic nephropathy (DN) in a nation-wide, population-based cohort with childhood onset type 1 diabetes.

Materials and Methods: Patients born after 1963, diagnosed with childhood-onset diabetes between 1 July 1977 and 31 December 2003 are reported to The Swedish Childhood Diabetes Registry (n=12 046). Using personal identification numbers the register was linked to The Swedish Registry for Active Treatment of Uraemia. Forty patients were identified with ESRD out of which 33 (82%) had DN. Median duration from onset of diabetes to start of active treatment for uremia (dialysis or transplantation) was 20 years (range 15–23). None of these patients had an onset of diabetes after 1985. Thus, all patients diagnosed with diabetes between 1 July 1977 and 31 December 1985 with diabetes duration longer than 15 years comprised the study cohort (n=4420). Cox proportional hazard analysis was

used to compare the risk of developing ESRD in different age at onset groups.
Results: After a median time of follow-up of 21 years (range 15–27) 33/4420 patients had developed ESRD. A significant difference in risk of developing ESRD (p=0.001) was found between three five-year strata of age at onset. No patient with onset of diabetes before 5 years of age had developed ESRD. When the two groups with onset of diabetes before the age of ten [0–4 and 5–9 years, (n=2420)], were pooled a significantly reduced risk of developing ESRD was found in the group with prepubertal onset of diabetes (p=0.003).
Conclusion: In this population-based Swedish cohort with childhood onset of diabetes after 1 July 1977 less than 1%, of the patients had developed ESRD at a median time of follow-up of 21 years. A prepubertal onset of diabetes seems to prolong the time to development of ESRD.

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EURODIAB study of early mortality in type 1 diabetes diagnosed in childhood

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Background and Aims: The study objective was to provide a contemporary picture of mortality in Europe in the years following type 1 diabetes diagnosed before the 15th birthday.
Materials and Methods: Ten population-based EURODIAB registers followed up 18,819 children diagnosed since 1989. Follow up was through linkage with population registers in most centres although some centres contacted doctors providing patient care. Over 125,000 person years of follow-up was obtained.
Results: In total there were 78 deaths during follow up, approximately twice as many as would have been expected from the national age/sex specific mortality rates in the various countries. However the standardized mortality ratio (SMR) defined as the ratio of observed deaths to expected deaths varied from under 1 to 4.7 in the different countries. There was little relationship between a country's SMR and its incidence rate, or between a country's SMR and its Gross Domestic Product (US$ per head of population), a measure of national prosperity. Over a third of deaths could be directly attributed to diabetes, while many of the remainder were due to accidents or violence. In some countries deaths attributable to the withholding of insulin were reported. Deaths without any cause that could be determined at forensic autopsy ("dead in bed") were also reported. Small numbers of deaths occurring close to the time of diagnosis continue to occur in most countries, but these were not included in this analysis since ascertainment of such cases may be incomplete.
Conclusion: There is still a significant excess mortality among type 1 diabetes mellitus cases diagnosed in childhood even before the onset of late complications. The variability in this excess mortality over different centres is wide and cannot be explained in any obvious way. Many of the deaths caused by diabetes in this age-group should have been avoidable.

Mortality follow up results in centres arranged by ascending order of incidence rate

Centre	Cases	Registration period	Observed deaths (O)	Person years	Expected deaths (E)	SMR =O/E (95% CI
Lithuania	1006	1989–2003	7568	15	5.2	2.9 (1.6, 4.7)
Bulgaria (Eastern)	443	1989–1999	4069	10	2.1	4.7 (2.3,8.7)
Hungary	1968	1989–2002	13432	6	4.6	1.3 (0.5, 2.9)
Austria	1989	1989–2002	14744	6	4.9	1.2 (0.5, 2.6)
Spain (Catalonia)	1806	1989–2002	13316	3	4.7	0.6 (0.1,1.9)
Germany (Düsseldorf)	764	1989–2001	3778	2	0.9	2.2 (0.3,8.0)
Iceland	151	1989–2004	1160	0	0.5	0.0 (–, –)
Denmark	2287	1989–2002	13104	12	3.3	3.6 (1.9, 6.3)
UK (N. Ireland)	1311	1989–2002	9458	10	3.2	3.2 (1.5, 5.8)
Sweden	7094	1989–2002	45158	14	9.7	1.4 (0.8, 2.4)
	18819		125787	78	39.1	

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Effects of insulin therapy on liver fat content and hepatic insulin sensitivity in patients with type 2 diabetes

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Background and Aims: To determine whether chronic hyperinsulinemia induced by exogenous insulin therapy changes liver fat content (LFAT) or hepatic insulin sensitivity in metformin-treated type 2 diabetic patients. We have previously shown that metformin alone does not change LFAT.
Materials and methods: 14 type 2 diabetic patients (age 51 ± 2 yrs, BMI 33.1 ± 1.4 kg/m^2 and HbA_{1c} 8.9 ± 0.3%) poorly controlled on metformin were treated with additional basal insulin for 7 months. Whole body and hepatic insulin sensitivity (6-hour euglycemic hyperinsulinemic clamp combined with [3-^{3}H]-glucose), LFAT (magnetic resonance proton spectroscopy), fat distribution (MRI) and fat free and fat mass (bioimpedance pletysmography) were measured before and after insulin treatment.
Results: The insulin dose averaged 75 ± 10 IU/day (0.69 ± 0.08 IU/kg, range 24–132 IU/day). HbA_{1c} decreased from 8.9 ± 0.3 to 7.4 ± 0.2% (p=0.0001). Whole body insulin sensitivity increased from 1.5 ± 0.3 to 2.0 ± 0.3 mg/kg· min (p=0.027). LFAT decreased from 17 ± 3 to 14 ± 3% (p=0.04) although body weight increased from 101.3 ± 5.8 to 104.3 ± 6.1 kg (p=0.02). Basal hepatic glucose production (HGP) decreased from 3.42 ± 0.20 to 2.66 ± 0.33 mg/kg ffm · min (p=0.03). Hepatic insulin sensitivity increased since HGP was better suppressed by insulin after than before insulin therapy (0.21 ± 0.19 vs. 1.04 ± 0.28 mg/kg ffm· min, p= 0.009). The % suppression of HGP by insulin increased from 72 ± 8 to 105 ± 11% (p=0.0013). 83% of the increase on body weight (3.0 kg) was attributable to increase in fat free mass from 69.5 ± 3.8 to 72.0 ± 4.1 kg, p=0.004. Fat mass and distribution remained unchanged. LFAT and insulin requirements correlated significantly (r=0.58, p=0.02) as did LFAT and serum ALT concentration (r=0.70, p=0.006).
Conclusion: Insulin therapy improves hepatic insulin sensitivity and slightly but significantly reduces liver fat content.

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Reduced intra-hepatic fat content is associated with increased lipid oxidation in patients with type 1 diabetes

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Background and Aims: Peripheral and hepatic insulin resistance may be associated with ectopic fat accumulation potentially determined by reduced lipid oxidation (LOx). We have previously shown that T1DM patients are characterized by moderate peripheral insulin resistance in association with higher intramyocellular lipid content. This study was undertaken to assess whether these patients are also characterized by intrahepatic fat (IHF) accumulation and impaired LOx.
Materials and Methods: 18 T1DM patients (6F/12M, age=35 ± 7 ys, BMI=23 ± 3 kg/m^2, HbA1c: 8.7 ± 1.4%) and 18 healthy matched individuals (NOR) were studied by means of 1) euglycemic-hyperinsulinemic clamp combined with [6,6-$^2H_2$]glucose infusion to assess whole body glucose metabolism, 2) indirect calorimetry to assess glucose and LOx 3) localized ^{1}H-MR spectroscopy of the liver to assess IHF content.
Results: T1DM patients showed reduced insulin-stimulated glucose metabolic clearance rate (MCR: 3.8 ± 1.8) in comparison with NOR (6.0 ± 1.6 ml/[kg min]; P<0.001). The endogenous glucose production was higher in T1DM patients (P=0.001) and its suppression was impaired during insulin administration (67 ± 31% vs. 90%8; P=0.02) in comparison with NOR. The IHF content was reduced in T1DM (1.4 ± 0.9%) in comparison with NOR (2.3 ± 1.1%; P=0.04) in association with a reduced respiratory quotient (RQ: 0.74 ± 0.05 vs. 0.82 ± 0.06; P=0.01) and increased fasting LOx (1.5 ± 0.5 vs. 0.9 ± 0.5 mg/[kg min]; P<0.01) and regardless metabolic control.
Conclusion: In T1DM patients peripheral and hepatic insulin resistance were not associated with increased IHF accumulation. In fact, T1DM patients showed a reduced IHF content which was associated with an

enhanced fasting LOx. This study demonstrates that in humans hepatic insulin resistance may be associated with a low IHF content and suggests that LOx may be more relevant than insulin resistance in the regulation of the IHF.

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Relationship between non alcoholic steatohepatitis (NASH) and insulin resistance and role of PPARγ2 and adiponectin polymorphisms in these metabolic disorders

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Background and Aims: The association between NASH and insulin resistance (IR) is well known, but a causal relationship has not been yet established. We have studied in the general population the relationship between NASH and insulin resistance and the influence of two polymorphisms: PPARγ 2 Pro12Ala and Adiponectine G-11391A on the outcome of NASH and IR.
Materials and Methods: A cohort of 5212 subjets (half men, half women), representative of the French general population (the DESIR study – Données Epidémiologiques sur le Syndrome d'InsulinoRésistance), aged 30–60 years, were studied for clinical, antropometric and biological data pertaining to IR at baseline and after 3 years. NASH was diagnosed if Aspartate Transaminase and/or Alanine Transaminase and/or Gamma Glutaryl Transferase values were above the mean +2SD of normal values and IR was defined as serum insulin within the upper fifth quintile of distribution. Subjects with alcohol consumption >40 g/day and those with positive viral hepatitis detection were excluded. PPARγ2 and adiponectin polymorphisms were genotyped using a polymerase chain reaction followed by hybridation with fluorescent probes.
Results: At baseline (T0), the subjects were divided in 4 groups: A/ without NASH or IR (n=3125); B/with NASH without IR (n=97); C/ without NASH with IR (n=660); D/with NASH and IR (n=65) (Khi squared test=53.322, p<0.0001). After 3 years, 17 subjects of group B had developed IR, vs 304 of group A: Odds Ratio (OR) = 1.972 (95% CI 1.153–3.374); p<0.012. In the mean time, 45 subjects of group C developed NASH, vs 113 of group A: OR= 1.95 (95% CI 1.365–2.786); p<0.0001. The allele frequencies were 20% for Ala of Pro12Ala of PPARγ2 and 15% for A of G-11391A of adiponectin. Between the subjects without NASH at T0, the Ala allele of PPARγ2 reduced the development of IR (OR= 0.71 (95% CI 0.50–1.00); p<0.046 adjusted for sex and age) after 3 years; the A allele of G-11391A of adiponectin reduced the development of NASH (OR= 0.516 (95% CI 0.30–0.88) p <0.012 adjusted for sex and age) after 3 years.
Conclusion: NASH is a risk factor for IR and vice-versa. The Ala allele of Pro12Ala of PPARγ2 protects from the outcome of IR. The A allele of G-11391A of adiponectin protects from the outcome of NASH.

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The association of adiponectin with insulin sensitivity and liver fat content depends on the amount of body fat and body fat distribution

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Background and Aims: In humans, low plasma adiponectin concentrations precede a decrease in insulin sensitivity and predict type 2 diabetes independently of obesity. However, whether the degree of adiposity or body fat distribution influence the association between adiponectin and insulin sensitivity (IS) is not known.
Materials and Methods: We investigated the association between plasma adiponectin levels and IS (OGTT, n=900 and euglycemic hyperinsulinemic clamp, n=299) in different ranges of percentage of body fat (PFAT) and waist-to-hip ratio (WHR) in cross-sectional analyses in a large cohort of normal glucose tolerant subjects. A group of 122 individuals had measurements of liver fat (LF) and a sub-group of 108 subjects had follow-up data.
Results: In cross-sectional analyses, plasma adiponectin concentrations, adjusted for gender and age, correlated positively with IS (OGTT and clamp, both p<0.0001) and negatively with PFAT (OGTT and clamp both p<0.0001) and LF (p<0.0001). However, the association of adiponectin with IS and LF depended on the magnitude of adiposity (p for interaction; IS: adiponectin*PFAT <0.0001 for OGTT and 0.002 for clamp; LF: p=0.01). Similar results were found for WHR. The association of adiponectin with IS and LF was stronger when subjects were obese and/or had more upper-body adiposity. Among factors (TNF-a, IL-6, FFA) regulating IS and/or plasma adiponectin concentrations only IL-6 affected the interaction adiponectin*PFAT (IS: p=0.03, OGTT and p=0.08, clamp; LF p=0.03).
In longitudinal analyses plasma adiponectin predicted change in IS (OGTT) only in obese (n=54, p=0.03) but not in lean (n=54, p=0.68) individuals.
Conclusion: In conclusion, these data suggest that adiponectin determines whole-body IS and LF particularly in individuals with high amount of PFAT and especially when it accumulates in the upper body. In lean subjects, IL-6 possibly antagonizes effects of adiponectin on IS. This may have an impact on intervention strategies to prevent a decline in IS.
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Assessment of adiponectin concentration in patients with nonalcoholic fatty liver disease

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Background and Aims: Although nonalcoholic fatty liver disease (NAFLD) is often observed in patients with obesity, hyperlipidemia and type 2 diabetes mellitus (DM), it shows insulin resistance independent of body mass index and glucose tolerance. Visceral fat deposit is strongly associated with insulin resistance and the development of fatty liver. Adiponectin enhances insulin sensitivity and protects against atherosclerosis, and lower adiponectin level is observed in patients with visceral obesity. Therefore hypoadiponectinemia may play an important role in metabolic disorders related to NAFLD. The aim of the study is to evaluate insulin resistance, glucose tolerance and adipocytokines, and especially to clarify the relationships between adiponectin and other parameters in patients with NAFLD.
Materials and Methods: 193 patients, diagnosed as NAFLD and with fasting plasma glucose level less than 126 mg/dl, participated in this study. Clinical features: 68 males/125 females, mean age 55 ± 12 years, BMI 26.1 ± 3.9 kg/m^2, waist-to-hip ratio (WHR) 0.86 ± 0.05, FPG 106 ± 10 mg/dl, HbA1c 5.4 ± 0.5%. All patients were divided in three groups, normal glucose tolerance (NGT: 62 patients, 21 m/41 f), impaired glucose tolerance (IGT: 81 patients, 28 m/53 f) and DM (50 patients, 19 m/31 f) according to a 75 g oral glucose tolerance test. Insulin resistance index (HOMA-IR, insulin sensitivity index or ISI, quantitative insulin sensitivity check index or QUICKI) and insulin secretion capacity (insulinogenic index and HOMA-β) were calculated. Serum lipids and adipocytokines such as adiponectin (monomer), leptin, TNF-α and PAI-1 were all measured under the fasting conditions. Control subjects (6 m/9 f) without NAFLD and glucose intolerance also received the same investigations.
Results: Serum adiponectin concentration was markedly reduced even in NGT group with NAFLD compared with control subjects (6.8 ± 2.5 vs 14.6 ± 6.4 μg/ml respectively, p<0.01). It was significantly higher in females than in males in all studied NAFLD groups: total patients 6.9 ± 3.0 (5.0 and 7.9, in males and females respectively), NGT 6.8 ± 2.5 (5.0 and 8.3), IGT 7.0 ± 3.1 (5.0 and 8.1), DM 5.9 ± 2.1 (4.8 and 6.7). Serum adiponectin level was positively correlated with HDL-cholesterol (r=0.390, p<0.0001), ISI (r=0.341, p<0.0001) and QUICKI (r=0.396, p<0.0001), and negatively correlated with waist circumference (r=-0.325, p=0.0001), WHR (r=-0.415, p<0.0001), triglyceride (r=-0.212, p=0.0146), fasting insulin concentration (r=-0.320, p<0.0001) and HOMA-IR (r=-0.334, p<0.0001). However no significant correlation was found between adiponectin and parameters such as hip circumference, HbA1c and insulinogenic index. Interestingly adiponectin had a negative correlation with PAI-1 (r=-0.310, p=0.0002) but no association with other adipocytokines such as leptin and TNF-α.
Conclusion: Serum adiponectin concentration was decreased in patients with NAFLD independent of glucose tolerance and associated with index of visceral obesity, insulin sensitivity, lipid profiles and PAI-1 concentration, suggesting that NAFLD has aspects of metabolic syndrome. Metabolic disorders observed in NAFLD may be related to hypoadiponectinemia.

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Insulin resistance and steatosis alter adiponectin receptor expression in liver

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Background and Aims: Type 2 diabetes and obesity is closely associated with non-alcoholic fatty liver disease (NAFLD), which ranges from simple fatty liver (FL) to fibrotic non-alcoholic steatohepatitis (NASH). Visceral adiposity causes insulin resistance and steatosis in the liver. Adipocyte-derived hormone, adiponectin, may play roles in insulin action and liver fibrosis. Recently, two adiponectin receptors (AdipoR1 and AdipoR2) were identified, the latter of which is predominantly expressed in the liver. We investigated the regulation of AdipoR expression in NAFLD and in hepatocyte cell line.
Materials and Methods: Liver biopsy samples were obtained from patients with normal liver (n=9), patients with FL (n=8) and patients with NASH (n=8). Mean HOMA-R in each group was 1.93, 4.91 and 3.60, respectively. Mean steatosis score was 0.7, 3.4 and 2.6, respectively. For in vitro studies, we used a simian virus 40 large T antigen immortalized normal human hepatocyte cell line T5b cells. AdipoRs mRNA expression was quantitated by using cDNA microarrays and real-time PCR.
Results: (1) cDNA microarray analysis revealed that AdipoR2 expression was significantly up-regulated in fatty liver (0.7, parametric P=0.000738, permutation P=0.0011) and NASH (0.8, parametric P=0.000135, permutation P=0.0001) compared with normal liver (0.5).
To investigate how AdipoRs are regulated in the condition of insulin resistance, we examine the effect of insulin and TNF-α on T5b cells. (2) Insulin dose-dependently (1–2 mg/ml) down-regulated the levels of AdipoR2 mRNA (at 2 mg/ml, 61.4 ± 11.4 arbitrary units (AU), vs. control 69.2 ± 10.2 AU, P<0.01), whereas TNF-α dose-dependently (0.1–10 ng/ml) up-regulated it (at 2ng/ml of TNF-α, 76.7 ± 9.8 AU, P=0.03). TNF-α reversed the insulin-induced decrease of AdipoR2 expression.
(3) A thiazolidinedione pioglitazone up-regulated the expression of AdipoR2 mRNA (at 2 mM, 81.5 ± 11.3 AU, P<0.0001). Pioglitazone additively increased TNF-α-induced AdipoR2 expression.
(4) AdipoR1 mRNA expression was not altered by treatment with insulin, TNF-α or pioglitazone.
To analyze gene expression during steatosis of the cells, we established in vitro fatty hepatocytes. Incubation of T5b with 0.5 mM free fatty acid (FFA, 2:1 oleate/palmitate) for 6 h increased cellular triglyceride contents 9.5-fold higher than control (1335.5 ± 76.5 mg/g·protein). (5) AdipoR2 mRNA level was decreased in fat containing T5b cells to 89.0 ± 2.6% (P<0.0001), whereas AdipoR1 mRNA was not altered.
Conclusion: Insulin down-regulates, and TNF-α up-regulates AdipoR2 mRNA expression in hepatocytes. Liver might compensate for excessive action of insulin and for TNF-α-induced insulin resistance by altering AdipoR2 expression. Pioglitazone up-regulates not only adiponectin expression in adipocytes, but also AdipoR2 expression in liver, suggesting that this agent improves insulin resistance and exerts anti-atherogenic property by up-regulating adiponectin-AdipoR system. Although AdipoR2 is down-regulated in FFA-induced steatosis of hepatocytes, it is paradoxically up-regulated in human fatty liver. Development of fatty liver involves complex factors in addition to excessive supply of substrates.

OP 27
Optimising diabetes care

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Patterns of care delivery is a stronger predictor of diabetes complications than the late diagnosis of diabetes

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Background and Aims: Diabetes is a chronic disorder that leads to premature morbidity and mortality through the development of specific microvascular and macrovascular complications. In a previous study we showed that the development of diabetes complications was associated with low levels of health care utilisation, HbA1c and HDL-cholesterol testing over a seven year period. However, we also found that patients who developed diabetes complications may have been diagnosed with diabetes later than those who remained free of these conditions, and we could not determine whether the pattern of care or late diagnosis was the stronger predictor of adverse diabetes outcomes. As this issue has significant implications for how diabetes is managed, we sought to resolve this question by conducting an epidemiological investigation.
Materials and Methods: The study used an extract from the Australian Medicare database. The extract comprised a national sample of patients with a diagnosis of diabetes in 1993 to 1994 (early diagnosed patients). Diabetes was identified using HbA1c as the index marker. The parameters tested included the major aspects of diabetes management related utilisation which were readily identifiable by the system through specific funding items. The main outcome measure was vision-threatening retinopathy, and the study variables included GP, medical specialist and optometrist attendances, and HbA1c and HDL-cholesterol tests.
We used a case-control study design. Cases were diabetes patients who received their first episode of laser photocoagulation therapy in 2000 (n = 665). Controls were a random sample of diabetes patients who had never received this treatment (n=1297). We extracted health care utilisation data for the period 1993 - 2000 for each of the groups.
Results: The mean age of controls was slightly older than cases (cases = 61.5 (sd = 13.0) years, and controls = 62.8 (sd = 13.7) years)). There was a greater proportion of men in each group (cases = 51.7% and controls = 52.5%), but there was no difference in gender between the groups (X^2 = ns). There were significant differences in health care utilisation. In each year cases were less likely to attend a GP than controls, from OR = 0.91 (p<0.0001), in 1993 to OR= 0.95 (p<0.0001) in 1999; less likely to attend a medical specialist, from OR = 0.82 (p<0.0001) in 1993 to OR = 0.86 (p<0.0001) in 1999; less likely to be tested for HbA1c, from OR = 0.79 (p<0.0001) in 1993 to OR = 0.59 (p<0.0001) in 1999. Annual trends showed that cases were more likely to utilise health services as they approached the first episode of laser therapy, although their levels of utilisation at no time reached that of controls. In a logistic regression analysis the strongest predictors for the development of vision-threatening retinopathy were annual optometry attendances and HDL-cholesterol testing over a seven year period. Whilst these indicators may be significant in their own right, they could also be important indicators of the overall quality of diabetes care.
Conclusion: We found that lower levels of diabetes management related health care utilisation was a stronger predictor of vision-threatening retinopathy than late diagnosis and confirmed our earlier findings. The study highlights the importance of structuring health delivery services and interventions at a national level to improve the management of diabetes for the prevention of diabetes complications.

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General practitioners focus more on microvascular complications in patients with newly diagnosed type 2 diabetes than risk factors for cardiovascular disease

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Background and Aims: Long term diabetic complications often engage both macrovascular and microvascular changes. The most common life threatening complication is cardiovascular disease (CVD). Although CVD in the general population is decreasing, patients with diabetes does not show similar trend. This could be a result of a more aggressive vascular disease among diabetes patients, but it could also be a result of doctors not

focusing on macrovascular disease among patients with diabetes. Our aim was to study if there were any differences in the general practitioners (GP) examination of macrovascular and microvascular complications in newly diagnosed diabetes patients.
Materials and Methods: One hundred and five (M/F 58/47, mean age 55.8 ± 6.6 years) patients with newly diagnosed diabetes were consecutively included in the Diabetes registry in the Västerbotten intervention programme (DIVE) from 1 January 2001–30 June 2003. A survey of the computerised medical records was performed, mean time of 1.5 ± 0.6 years after diagnosis of diabetes. It was noted whether the GP had examined signs of microvascular or macrovascular disease according to guidelines for diabetes care published by The National Board of Health and Welfare, Sweden, 1999. Results of these examinations and if any diagnosis or comment on these results was found in the medical record were registered.
Results: 93 of 105 patients were diagnosed with type 2 diabetes (T2DM), 11 with unclassified diabetes and one patient with secondary diabetes. 66.4% (95% CI 57.4–75.4) of the patients were examined for retinopathy while 93.0% (95% CI 88.1–97.9) had a spot urinary sample examined for protein excretion. An ECG was taken in 33.7% (95% CI 24.7–42.7) of the patients. No gender differences were found in the proportion examined for long term complications. Retinopathy was found in 4/67 (6%) examined. Nephropathy was diagnosed in the medical record of 4 (3.8%) patients although 13 (14.0% of examined) showed trace or more of protein in a urinary sample. In 16 patients (18.4%) CVD was diagnosed.
Conclusion: In this study of middle aged patients with newly diagnosed diabetes there was a significant trend that a greater proportion was screened for microvascular complications than for macrovascular disease. Our findings implicate that the GPs are more focused on microvascular than macrovascular disease in newly diagnosed T2DM despite the knowledge that cardiovascular disease is far more common in these patients, which was also found in our study. Praxis studies, like our, of ordinary clinical care for diabetes patients might highlight the need of further improvement of education and application of guidelines.

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Evaluation of an endocrinologist-led family physician-run multidisciplinary diabetes care plan

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Background and Aims: Newly diagnosed patients with type 2 diabetes mellitus require comprehensive assessment, medical stabilization and intensive education at the onset of illness to facilitate their long-term care. An integrated patient care plan was established to link up the services of endocrinologist, family doctors, nurse educator and dietitian, with the aim to empower these patients in self-management after a short but intensive 3-month programme.
Materials and Methods: Newly diagnosed type 2 diabetes patients who were referred to public health services of New Territories West were enrolled into the programme. The care plan consists of 3 visits to the family physician, individual and group counseling by diabetic nurse and dietitian, and case review with the endocrinologist. Programme was evaluated for its effectiveness by comparing subjects' Knowledge, Attitude and Practice (KAP) scores, metabolic control, clinical and behavioural outcomes before and after intervention..
Results: Between February 2004 to February 2005, seventy-five patients (58.7% males, age range 23 to 85 years) completed the care plan. The mean HbA1c decreased from 8.7 ± 2.0 at baseline to 7.0 ± 1.0 on discharge (paired t-test, $p<0.001$). While only 40% had HbA1c ≤ 8% pre-, 84% achieved this target post intervention (Chi square, $p<0.0001$). Patients' mean Knowledge (K), Attitude (A) and Practice (P) scores increased significantly from 14.8 ± 4.6 to 19.0 ± 2.2 (paired t-test, $p<0.0001$), 1.8 ± 1.0 to 2.0 ± 0.9 (paired t-test, $p<0.02$) and from 4.9 ± 2.2 to 6.4 ± 1.5 (paired t-test, $p<0.0001$) respectively. 93% of the diabetics could return to primary health care for further management while 7% required specialist care. On completion of programme, 48% of patients were monitoring their blood glucose at home.
Conclusion: An endocrinologist-led family physician-run multidisciplinary diabetes care plan was effective in assessment, empowering self-management and maximizing initial medical treatment of patients with newly diagnosed type 2 diabetes mellitus.

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A unique public private partnership for primary prevention of diabetes and cardiovascular disease at the worksite: a collaboration of CDC, GE Power and the National Business Group on Health

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Background and Aims: The worksite represents a unique opportunity for diabetes education and prevention, which can positively impact productivity. To address this issue, the National Diabetes Education Program (NDEP), a joint CDC and NIH program, through its Business and Managed Care Workgroup (BMC), has developed a web based tool for businesses called diabetesatwork.org. The target audiences include: large and small businesses, unions, occupational health professionals, public health agencies, managed care groups, non profit organizations, wellness and benefits personnel. This tool has lesson plans that can be adapted for on site education. NBGH and the multinational GE Power partnered with CDC DDT to develop a program for employees at high risk for developing diabetes and cardiovascular disease and is in the process of measuring outcomes and ROI (Return on Investment). GE Power can serve as a model for other businesses.
Materials and Methods: Identify and evaluate the populations (participants vs control group) through serial cardiovascular HRAs (Health Risk Assessments) at multiple worksites. Adapt the diabetesatwork.org lesson plans to complement its own program, **0, 5, 10, 25 (**0 smoking, 5 fruits/vegetables/day, 10 000 steps/day, BMI <25) and its Health Coach Program. Monitor participants' survey on lifestyle changes (dose response based on % of activities attended). Monitor health care costs, utilization, and changes in HRA health status during periods of: Pre-intervention, intervention, post intervention (6, 12, 18 month intervals). Costs included: health plan claims data, prescription costs, disability cost data, utilization data (admissions, ER visits), disability days
Results: Pre and post HRA monitors: Total cholesterol(mg/dl): –3.1, p=0.000, HDL(mg/dl): +1.6, p=0.000, LDL(mg/dl: –4.0, p=0.000, triglycerides: –7.7, p=0.039, serum glucose(mg/dl): –5.4, p=0.000, systolic blood pressure(mmHg): –2.5, p=0.000, diastolic blood pressure: –0.7, p=0.012, BMI (kg/m^2): –0.0, p=0.507, waist circumference (cm): –0.1, p=0.412. Change in predicted risk of primary cardiac event (cardiac event = fatal, non fatal MI, sudden death in surgical intervention) = **Mean change** in real 5 year CHD risk: –0.002, p=0.001. **Mean change** in real 10 years risk: –0.003, p=0.003. **Mean change** in 5 year risk (age held constant): –0.004, p=0.000. **Mean change** in 10 year risk (age held constant): –0.008, p=0.000. Per 1000 employees screened, 4 events in 5 years are averted. 24.8 events averted in the screened population At $40,000/event =$992,000
Conclusion: Worksite education interventions represent excellent opportunities for primary prevention of diabetes and cardiovascular disease in identified high risk populations. Public and private partnerships can accomplish some of these interventions.

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Electronic food-list improves the metabolic control in type 1 diabetes mellitus

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Background and Aims: Substantial differences between pre-and postprandial glucose levels, which are critical for the metabolic control in type 1 diabetes mellitus (DM1) patients treated by insulin pump are often caused by an inappropriate insulin bolus.
The aim of our study was to evaluate the efficacy of the electronic food-list Diatron (JAVA application for cell phones) on the assessment of carbohydrate unit volume and on the calculation of the optimal bolus amount. Diatron can be easily installed on any cell phone supporting JAVA applications and covers more than 1 500 sorts of food with their nutrition values (carbohydrates, proteins, fat, glycaemic index and energy status), it is easy to use and does not require any additional devices.
Materials and Methods: Twenty four patients (from 25) treated by insulin pump (using insulin analogues) completed the study. Mean age of the patients was 39.4 (± 12.8) years, mean duration of DM1 was 16.3 (± 9.1) years and the mean time of the treatment by the insulin pump was 2.2 (± 1.7) years. The study lasted 2 months. Patients regularly monitored their glucose levels: two preprandial values and two values 1.5 hour after the meal. In the 2nd month the patients used the electronic food-list Diatron for the carbohydrate counting and assessed the appropriate bolus amount according to their individual insulin sensitivity. In all patients the glucose

monitoring was performed by the same type of personal glucometer. We evaluated the level of hemoglobin HbA1c, the mean preprandial and postprandial glucose levels, the differences between post- and preprandial glucose levels, the lability index, the body weight, the body mass index (BMI), the blood pressure values, bolus amounts and the daily basal/bolus proportion.
Results: The mean value of hemoglobin HbA1c before starting the study was 6.6 (± 1.1%). After the 1st month (self-monitoring 4 values daily) the mean value of hemoglobin HbA1C dropped to 6.2 (± 1.1%) ($p < 0.001$) and after the 2nd month (self-monitoring 4 values daily + use of the electronic food-list Diatron) to 5.7 (± 1.1%) ($p < 0.001$). The mean bolus amount increased significantly in the 2nd month and the daily basal/bolus proportion shifted towards a higher bolus portion. In addition, the postprandial glucose levels, the differences between post- and preprandial glucose levels and the lability indexes dropped significantly in the 2nd month. We did not find differences in the mean preprandial glucose levels, body weight, BMI and blood pressure. The postprandial hypoglycaemia (glycaemia <3.3 mmol/L) occurred in 2.2% of measured values in the 1st month and in 3.1% in the 2nd month, respectively.
Conclusion: The use of a simple, available and affordable device for the proper assessment of carbohydrate unit amount and ensuing optimal dosage of the insulin bolus are of great importance for improving the metabolic control in type 1 diabetes mellitus patients.

	1st month	2nd month	p
Preprandial glycaemia (mmol/L)	7.2 ± 3.2	7.2 ± 3.1	n.s.
Postprandial glycaemia (mmol/L)	9.8 ± 3.7	7.9 ± 3.3	$p < 0.001$
Bolus amount (UI)	4.7 ± 2.8	5.2 ± 2.9	$p < 0.001$
Post-preprandial glycaemia difference	3.6 ± 2.8	2.1 ± 1.7	$p < 0.001$
Daily basal/bolus proportion (UI/day)	0.62 ± 0.06	0.60 ± 0.06	$p < 0.01$
Lability index ((mmol/L)²/hour/week)	354 ± 139	125 ± 55	$p < 0.001$

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The role of titration enforcement for the treat-to-target concept with add-on insulin therapy in patients with type 2 diabetes
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Background and Aims: Evidence suggests that good glycaemic control can help prevent or delay the onset of late complications associated with type 2 diabetes. One treatment option for patients with advancing type 2 diabetes who are failing on therapy with oral antidiabetic agents is to add on insulin glargine; however, some effort is required to realize the full potential of this therapy. This analysis compares five successive multicentre, randomized, open trials in patients with type 2 diabetes receiving insulin glargine plus oral agents to determine the effect of a forced titration schedule and intensified titration monitoring on glycaemic control.
Materials and Methods: The analysis includes blinded data from an ongoing trial (Trial 5) comparing treatment with oral agents plus either the long-acting basal insulin, insulin glargine or the short-acting insulin analogue insulin lispro. While there was no supervision of titration in Trial 1, target fasting blood glucose (FBG) levels were strictly enforced in Trials 2-5 (FBG levels ≤5.5 mmol/L [≤100 mg/dL] for basal insulin; preprandial blood glucose ≤5.5 mmol/L [≤100 mg/dL] and postprandial blood glucose ≤7.5 mmol/L [≤135 mg/dL] for the short-acting insulin in Trial 5) and titration was monitored by direct contact with the physician and/or diabetes educator.
Results: Details related to the monitoring and HbA_{1c} levels at study endpoint (Trials 1-4; Trial 5 ongoing) are presented in the table.
Conclusion: Titration enforcement by additional visits or weekly calls enables the majority of patients on oral agents plus basal or prandial insulin to reach HbA_{1c} levels close to 7%. Furthermore, insulin glargine titration to FBG levels ≤5.5 mmol/L (≤100 mg/dL) can result in HbA_{1c} levels below 7% and intensified monitoring of the insulin dose may assist patients in achieving target glycaemic control.

Trial	1 (Study 4001)	2 (Study 4009)	3 (Treat-to-Target)	4 (LAPTOP)	5* (APOLLO)
N	458	616	367	176	124 (of 418)
Year	1999–2000	2000–2001	2000–2001	2001–2003	2003–ongoing
Duration (weeks)	24	24	24	24	44
Titration monitoring	Questionnaire in case report form	Direct investigator contact. FBG and insulin dose submitted to coordinating centre by fax	Direct investigator contact. FBG and insulin dose submitted to coordinating centre by fax	Direct investigator contact. FBG and insulin dose submitted to coordinating centre by electronic data capture (EDC)	Direct investigator contact. FBG and insulin dose submitted to coordinating centre by EDC. Additional weekly calls to adjust insulin dose, if HbA_{1c} levels were >7%
Achieved HbA_{1c} (%)	7.99	7.20	7.00	7.15	6.76

*Blind data monitoring (pooled treatment groups)

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OP 28
Incretin action

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The two intestinal incretins differentially regulate glucagon secretion due to differing intraislet paracrine effects

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Background and Aims: The incretins, Glucagon-Like Peptide-1 (GLP-1) and Glucose-dependent Insulinotropic Peptide (GIP), stimulate insulin (Ins) secretion, but for unknown reasons differ with respect to glucagon (Glu) secretion. In order to learn more about this and the intraislet paracrine relationships we performed the following experiments.
Materials and Methods: We used isolated and perfused porcine pancreases (n = 32) and infused GLP-1 and GIP (0.1, 1 or 10 nM) alone or together with excess amounts of neutralizing Ins or somatostatin (SS) antibodies (ab) under normal (arterial-to-venous) or reversed (venous-to-arterial) flow. We also infused (10 nM) Ins and Glu. The effluent was measured for Ins, Glu and SS.
Results: Compared to basal secretion (bs), SS-ab increased Ins secretion ($p = 0.017$) while Ins-ab decreased Glu secretion ($p < 0.001$) and stimulated SS secretion ($p = 0.003$). Reversed flow decreased Ins secretion ($p = 0.003$). Further addition of SS-ab increased Glu and Ins secretion, both $p < 0.05$. Ins infusion inhibited ($p < 0.001$) while Glu infusion stimulated ($p = 0.004$) SS secretion. Infusion of GLP-1 and GIP stimulated Ins (at 1 nM to 218 ± 29% and 188 ± 22%, respectively, both $p < 0.001$ compared to bs) and SS secretion (at 1 nM to 373 ± 49% and 179 ± 8%, respectively, both $p < 0.001$ compared to bs). GLP-1 inhibited ($p < 0.001$) Glu secretion (at 1 nM to 40 ± 3% of bs) while GIP had no effect. SS-ab addition reduced ($p = 0.020$) the effect of GLP-1 on Glu (to 72 ± 11% of bs). Ins-ab addition reduced ($p = 0.002$) the effect of GLP-1 on Glu (to 68 ± 5% of bs) and increased ($p = 0.032$) the effect of GIP on SS (to 284 ± 34% of bs). Reversed flow reduced ($p = 0.001$) the effect of GLP-1 on Glu (to 78 ± 7% of bs). Further addition of SS-ab completely abolished ($p = 0.01$) the inhibitory effect of GLP-1.
Conclusion: Our findings suggest that blood flows from the Ins cells to both Glu cells and SS cells. Both Ins and SS may inhibit Glu secretion while Glu stimulates SS secretion. GLP-1 stimulates both Ins and SS cells, while GIP stimulates Ins and Glu cells. We suggest that GLP-1inhibits Glu indirectly (via both Ins and SS) while GIP stimulates SS via Glu. This explains that both peptides increase Ins and SS secretion whereas only GLP-1 inhibits Glu.

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GLP-1, but not GIP, inhibits glucagon secretion via somatostatin in the perfused rat pancreas

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Background and Aims: The antidiabetogenic effect of glucagon-like peptide 1 (GLP-1) is not only based upon its potent insulinotropic actions, but also upon its ability to restrain pancreatic glucagon release. Interestingly, the only other identified incretin hormone apart from GLP-1, glucose-dependent-insulinotropic peptide (GIP), seems to exhibit -in spite of the many functional and structural similarities the two incretin hormones share- exactly the opposite effect by stimulating glucagon secretion. The underlying mechanism of this contrary mode of action has not been elucidated.
However, it is well established that both exogenous and endogenous somatostatin (SS) strongly inhibits glucagon secretion. It was therefore the aim of our study to evaluate a possible implication of SS in the GLP-1-induced inhibition and the GIP-induced stimulation of glucagon release. Furthermore, we were interested in examining the glucose-dependency of these effects, since the insulinotropic effects of both GLP-1 and GIP are strictly dependent on high to normal glucose concentrations.
Material and Methods: The pancreases of female Wistar rats were in situ perfused at a glucose concentration of 1.5 mM. In order to eliminate the biological actions of endogenous somatostatin (SS) an immunoneutralizing monoclonal antibody was administered and the insulin, glucagon and SS responses to 20 mM glucose, 10 nM GLP-1, 10 nM GIP and 0.1 and 10 nM SS (n=8 animals) were compared with those obtained from control experiments, in which no SS antibody was applied (n=6).
Results: As expected, the insulinotropic effect of both GLP-1 and GIP on insulin secretion was abolished under hypoglycaemic conditions, whereas glucagon secretion was strongly influenced by both GLP-1 and GIP. SS dose-dependently suppressed glucagon secretion to 79.6 ± 11.5% (0.1 nM – n.s.) and 35 ± 4.5% of basal level (10 nM – p=0,007), whereupon the effects of 0.1 nM, but not 10 nM SS could be completely eliminated by administration of SS antibody.
Accordingly, GLP-1 induced a significant inhibition of glucagon secretion by reducing it to 44.1 ± 2.6% (SEM) of basal level (p=0.0006). When the SS antibody was applied, GLP-1 caused still a restraint of glucagon secretion (66.6 ± 7.1% of basal level p=0.02), but this inhibition was significantly attenuated in comparison to the one achieved in the absence of SS antibody (p= 0.006).
On the contrary, GIP provoked a powerful stimulation of glucagon secretion irrespective of the absence or presence of SS antibody (178.3 ± 30.5% p=0.051 and 178.2 ± 21.7% of basal level $p < 0.05$). No significant difference between the two glucagon responses could be detected.
Conclusions: Even under hypoglycaemia, GLP-1 suppresses potently glucagon output in the perfused rat pancreas. Since immunoneutralization of endogenous SS results in a significant attenuation of the inhibitory effect of GLP-1 on glucagon secretion, we conclude that SS is involved in the regulation of glucagon secretion. However, the GLP-1-induced restraint is not completely abolished, giving rise to the assumption, that, in addition to the effects mediated via SS, also direct effects of GLP-1 apply.
Concerning the GIP-induced stimulation of glucagon secretion, SS does not seem to be involved, as no differences between the glucagon responses to GIP with or without SS antibody could be observed.

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The biological action of the GLP-1 analogue liraglutide is mediated by the activation of the TGF-β pathway in cultured human islets

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Background and Aims: Liraglutide is a long-acting analogue of GLP-1, a naturally occurring peptide hormone that has been shown to be capable of promoting proliferation, differentiation and inhibit apoptosis of pancreatic islets. The aim for this study is to investigate the signal pathways by which liraglutide regulates gene expression in pancreatic islets.
Materials and Methods: Isolated human islet preparations obtained from 3 independent donors were treated with liraglutide (10 nmol) for 22 hours, and the RNA extracted from islets was converted to cDNA and transcribed into cRNA. Individual cRNA preparations were then hybridized to U133A microarrays carrying approximately 23,000 genes, and the expression of genes in TGF beta pathway between liraglutide treated islets group (6 chips) and vehicle-treated islets (6 chips) was analyzed using GeneSpring software. Differentially expressed genes were selected by three methods: T-test ($P < 0.05$), a density score greater than 100 for positively identified gene, and the detection of more than 1.5 folds differences from control. Real-time PCR and western blot were performed to validate results obtained by microarray analysis.
Results: A comparison of liraglutide treated and vehicle-treated islets identified 287 genes that were differentially expressed between two groups (T-test, $P<0.05$). As far as the main molecules involved in the TGF-beta pathway, some of them (ERK1/2, TAK1, JUK1/2 and HPK1 etc) were not detected in any of the two treatment groups; the expression of some related genes (such as TGF beta Type ll R, PTK, C-Raf1 and CREB2 etc) did not show any regulation by liraglutide. Significantly, several key genes in TGF-beta pathway were up regulated in liraglutide-treated islets. Those included Ras (6.0 folds), PR53 (3.9 folds), MEK1/2 (4.56 folds), Smad1/5/8 (1.91 folds) and Smad 2/3 (3.48 folds). On the other hand various genes were down regulated by liraglutide. Those include TGIF (-3.21 folds), Smad6 (-2.67 folds), Smad7 (-1.50 folds) and Smurf 2 (-2.05 folds).
Conclusion: The TGF-beta pathway is involved in regulation of gene expression in human islets cultured in the presence of the GLP-1 analogue Liraglutide.
Support: Novo Nordisk

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Hedgehog signaling is involved in the differentiation of pancreatic ductal cells into insulin-secreting cells by GLP-1

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Background: The intestinal hormone glucagon-like peptide-1 (GLP-1) has been shown to promote an increase in pancreatic beta-cell mass via differentiation of non-insulin-secreting cells, but the mechanism(s) regulating this process remains unclear. The intercellular signaling molecules known as hedgehogs (Hhs) are critical regulators of development. Multiple Hhs have been identified in vertebrates, including Sonic hedgehog (Shh), Indian hedgehog (Ihh), and Desert hedgehog (Dhh). The activation of the hedgehog signaling pathway has been shown to be involved in the process of pancreatic organogenesis.
Aim of the Study: The aim of the present study was to investigate the regulation of hedgehog signaling molecules in the process of ductal cell differentiation induced by GLP-1.
Methods: Both in vivo and in vitro experiments have been conducted. Zucker diabetic rats were subjected to a 48 h infusion of human GLP-1 (30 pmol/kg per min), four days after the removal of the infusion pumps, rats were sacrificed and the pancreas harvested to investigate the expression of hedgehog signaling molecules by RT-PCR and immunoassaying. Rat pancreatic ductal ARIP cells were cultured in the medium containing 10 nM GLP-1 for 72 hours. RT-PCR was carried out with the total RNA extracted from treated cells. The expression of Hedgehog signaling molecules was investigated by RT-PCR and immunostaining techniques. Exendin 4 (a long acting analog of GLP-1), exendin 9 (a reverse agonist of GLP-1) and Cyclopamine (inhibitor of hedgehog signaling pathway) were used to validate the specificity of the data obtained. A mouse insulinoma cell line (MIN6) and a rat myoblast cell line (L6) were used as control for beta cell and non-beta cell-specific markers.
Results: Ex vivo immunostaining showed that the metabolic changes observed in rats treated with GLP-1 were associated with a decreased expression of SHH and an increase in IHH and PDX-1 in pancreas. After 72 hours of GLP-1 treatment, the expression of Shh in ARIP decreased, the expression of Ihh and Dhh increased. No obvious changes in the expression of PTC and PDX-1 were observed. A similar regulation of molecules induced by GLP-1 treatment was observed with cells treated with exendin 4, and this process was inhibited by the exposure of cells to either exendin 9, or cyclopamine.
Conclusion: In vivo and in vitro studies demonstrate that GLP-1 induces differentiation of pancreatic ductal cell into insulin secreting cells via the Hedgehog signaling pathway.

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GLP-1 induces subcellular re-distribution of PKC isoforms in rat skeletal muscle

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Background and Aims: GLP-1, like insulin, stimulates glucose transport, glycogen synthase *a* activity and glycogen synthesis in rat and human skeletal muscle tissue or cells. In this tissue, GLP-1 also increases the activity of several protein kinases – PI3K/PKB, p70s6 k, p44/p42 MAPKs –, and some of them, including PKC, have been proposed to mediate its stimulatory effect upon the hexose uptake and metabolism. As certain PKC isoforms have been implicated in the insulin-induced glucose transport, we have investigated the time-relationship of the subcellular distribution of PKC-α, -λ and -ε isoforms induced by GLP-1 in rat skeletal muscle, compared with that by insulin.
Materials and Methods: Paired *soleus* muscle from Wistar rats (two per rat) were incubated during 1 to 15 min in KRB containing 5 mM D-glucose and 1% BSA, in the absence (control) and presence of 10^{-10} M GLP-1 (maximal effective dose) or 10^{-9} M insulin. Then, cytosol and membrane fractions were prepared by differential centrifugation and subjected to immunoblot analysis, by using specific antibodies for each PKC isoform in particular. Data (mean±SEM) are referred to the control value at any given time of incubation.
Results: In muscle samples from 4–9 rats, GLP-1 provoked a rapid (1 min) trend towards an increase in membrane content of PKC-α, which reached statistical significance (p=0.011) at 3 min incubation (135 ± 11% of control), and continue to increase at 5 min (169 ± 29%, p<0.0001 and p=0.007 *vs* 1-min); that was followed by a decrease at 15 min (93 ± 14%); concomitantly, at 15 min, an increase in the cytosolic fraction was detected (147 ± 13%, p<0.0001 and p=0.015 *vs* 5-min); with insulin though, the maximal value in the membrane PKC-α was found at 3 min (168 ± 20% of control, p<0.0001 and p<0.005 *vs* 1-min), followed by a nadir at 5 min (82 ± 15%), with an apparent secondary increase at 15 min. GLP-1 also induced an increase in the membrane PKC-λ although later than that induced upon the α isoform, reaching at 5 min 155 ± 29% control (p<0.0001 *vs* control and p<0.0001 *vs* 1-min), and that was followed by a decrease at 15 min (116 ± 20%, p=0.021 *vs* min 5); with insulin, the maximal increase in membrane PKC-λ was already at 3 min (142 ± 14%, p<0.0001 *vs* control and p=0.005 *vs* 1-min), followed by a nadir at 5 min (94 ± 6%, p=0.001 *vs* 3-min) and, subsequently, by a secondary increase at 15 min (145 ± 14%, p=0.001 *vs* control and *vs* 5-min). In this group of experiments, however, membrane PKC-ε was not apparently modified by GLP-1 or insulin between 1 and 15 min.
Conclusion: These results suggest that PKC-α and PKC-λ isoforms could be implicated in the mechanism of the known stimulatory effect of GLP-1 upon glucose transport and metabolism in the skeletal muscle.

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Glucose uptake and kinases activity in adipose tissue, in response to GLP-1 and exendins in morbid obesity

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Background and Aims: GLP-1, Ex-4 and its truncated form Ex-9, both structurally related with GLP-1, stimulate, like insulin, glucose transport in fat and other rat and/or human extrapancreatic tissues. In the increasing effect of GLP-1 and both exendins upon glucose uptake in rat adipocytes, several kinases have been shown to be implicated. In muscle cells from morbidly obese and in type 2 diabetic patients, an altered response of some kinases has been proposed to account for the impaired glucose uptake by insulin. In this work we have studied the GLUT4 expression, and the effect of GLP-1, Ex-4, Ex-9 and insulin upon glucose transport and activity of some kinases, in adipocytes from morbidly obese patients.
Materials and Methods: Adipocytes were isolated by enzymatic digestion from subcutaneous fat tissue obtained, previously informed consent given, from 16 morbid obese patients undergoing bariatric surgery (10F/6M; age: 40 ± 3 yr; BMI: 50.1 ± 1.6 kg/m^2; fasting plasma glucose: 98.7 ± 4.5 mg/dl; HDL: 43.2 ± 4.3 mg/dl; cholesterol: 195.8 ± 14.0 mg/dl and triglycerides: 132.6 ± 9.6 mg/dl); for purpose of comparison of basal values, 11 normal subjects (7F/4M; age: 40 ± 3 yr; fasting plasma glucose: 87.9 ± 3.1 mg/dl), undergoing inguinal hernia surgery, were included. We measured glucose transport – 2-deoxi-D-[1,2-^{3}H]glucose uptake – after incubation without (basal) and with 10^{-12}–10^{-9} M GLP-1, Ex-4 or Ex-9 and 10^{-9} M insulin. The activity of PI3K – PIP_2 to PIP_3, by TLC –, PKB, p42/44 MAPKs and p70s6 k – Western blotting – was measured after 3 min without (basal) and with 10^{-9} M either peptide. GLUT-4 protein and mRNA were assayed by Western and Northern blot, respectively. Significances of results (mean ± SEM) were assessed by ANOVA test.
Results: In adipocytes from obese patients, GLUT-4/β-actin mRNA ratio (0.395 ± 0.052, n=4) was not different (p>0.5) from that in the control group (0.460 ± 0.086, n=4) nor was the protein content, this averaging 81.9 ± 14.1%, n=11, of normal subjects (100.0 ± 15.9%, n=6). In cells from 5 patients, basal glucose uptake (17.1 ± 1.4 fmol/10^5 cells) was increased (p<0.005) by 10^{-9} M insulin to 184 ± 14%; GLP-1, at 10^{-12} M, enhanced (p<0.05) the glucose uptake (126 ± 9%), but the effect faded out (p<0.025) in the 10^{-11} to 10^{-9} M range; yet, both Ex-4 and Ex-9 inhibited (p<0.05 or lower) the basal value. In cells from 3–4 patients, basal PI3K activity was increased (p<0.04 or lower) by all agents tested, with an overall mean value of 185 ± 13%; the same was detected in relation to p42 and p44 MAPKs (p<0.05 or lower, in all cases); PKB was only affected by insulin (235 ± 38%, p<0.0001), although the overall increment by GLP-1, Ex-4 and Ex-9 averaged 153 ± 17% (p<0.001). All peptides failed to modify p70s6 k phosphorylation. The overall basal activity of the five kinases was lower (p<0.0001) in obese patients than in normal subjects (50 ± 5% *vs* 100 ± 8%).
Conclusion: In adipocytes from morbidly obese subjects, GLP-1 and both exendins, unlike insulin, do not seem to clearly stimulate glucose uptake. The enhancing effect of GLP-1, Ex-4 and Ex-9 upon the activity of some kinases may be correlated with other actions of these peptides, as it is that upon the lipid metabolism, whose effects in adipocytes of obese patients have been reported.

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Exocytosis and ion channels in beta cells

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Regulated insulin release requires chloride channel 3 (ClC3) for proper granule acidification and priming

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Background and Aims: Uptake of chloride ions (Cl^-) via ClC3 channels in the insulin granule membrane may be important for the insulin granules gaining release competence, by regulating acidification of the insulin granules. We have investigated this possibility in mice with general knockout of ClC3 (ClC3-KO), in mice with conditional ClC3-ablation in pancreatic beta-cells (beta-ClC3-KO), as well as in insulinoma (INS-1) cells after ClC3 knock-down by RNAi.
Materials and Methods: In general ClC3-KO mice and their wildtype littermates were investigated by the following procedures: In vivo glucose challenges (11.1 mmol/kg i.p.), with p-glucose and p-insulin measured in parallel; Static batch incubations of pancreatic islets with insulin and glucagon release assayed by RIA; Single beta cell exocytosis monitored as increases in cell capacitance. Semiliki Forest Virus-based ClC3 gene transfer was used to test 'gain-of-function' in single ClC3-KO cells; and, insulin granule pH was monitored semi-quantitatively as changes in Lysosensor DND-189 fluorescence. In the conditional beta-ClC3-KO, pancreatic hormone release in batch incubations, and single-cell exocytosis was compared with wt. In INS-1 cells with ClC3 knock-down by RNAi, regulated release was monitored by human growth hormone (hGH) release detected by ELISA. The subcellular localization of ClC3 in insulin granules was detected by immunohistochemistry.
Results: 1) The general ClC3-KO mice group: The *in vivo* experiments revealed that 8 min after the glucose challenge, p-insulin increased by 96% in wt mice, whereas in the $ClC3^{-/-}$ mice, p-insulin remained unchanged (4% increase; $P<0.01$; $ClC3^{-/-}$ mice vs. wt mice). In isolated $ClC3^{-/-}$ islets, insulin secretion elicited by glucose (20 mM) was decreased by 72% ($P<0.001$ vs. wt). Ca^{2+}-evoked exocytosis in $ClC3^{-/-}$ B-cells was overall reduced by 80% ($P<0.001$ vs. wt). ClC3 gene transfer using the Semiliki Forest Virus expression system, partially rescued exocytosis in the $ClC3^{-/-}$ B-cells, which averaged 61% of that in wt B-cells ($P<0.001$; non-transduced vs. transduced $ClC3^{-/-}$ beta-cells). The reduction in exocytosis in $ClC3^{-/-}$ beta cells coincided with a reduced capacity for adjusting intragranular pH; 2) Conditional beta-ClC3-KO mice: glucose-evoked insulin secretion was reduced by 52% compared to wt ($P<0.001$; $n=12$ in both groups). Exocytosis evoked by a train of depolarisations was 64% suppressed ($P<0.001$; $n=19$ and 22, in wt and beta-ClC3-KO, respectively). 3) The silencing cell group: three designed siRNAs reduced hGH release by 74%, 86% and 76% compared to the non-silencing cells, respectively. In normal INS-1 cells, the subcellular localization of the ClC-3 protein was investigated by electron microscopy combined with immunogold labelling, which revealed that ClC3 primarily localizes to the insulin granule membrane.
Conclusion: ClC3 gene ablation results in a severe reduction in glucose-stimulated insulin secretion *in vivo* as well as *in vitro*. This reduction is due to defective single-cell insulin exocytosis, accompanied by an aberrant granule pH regulation. These results support the view that granular ClC3 channels are involved in making insulin granules release-competent.
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Depletion of cholesterol using a low concentration of methyl-beta-cyclodextrin reduces the exocytotic response in single mouse pancreatic B-cells

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Background and Aims: SNARE-proteins take part in the exocytotic process of many cell-types. Resent studies have shown a decreased expression of SNAP25 and syntaxin 1 in B-cells from GK-rats and diabetic patients, proposing an important role of these proteins in normal insulin secretion. It has been suggested that exocytotic proteins are clustered at the plasma membrane. The clustering occurs in areas rich of cholesterol distinct from lipid rafts and is essential for efficient fusion. The aim of this study was to investigate the importance of cholesterol-rich domains for insulin secretion.
Materials and Methods: Western Blot analysis preceded by sub-cellular fractionation on INS-1 cells and confocal microscopy on single primary mouse B-cells was performed to investigate the localization of the SNARE-complex. Insulin was measured on mouse islets using RIA. Exocytosis was studied on single mouse B-cells by capacitance measurements using the whole-cell configuration of the patch-clamp technique. Ultrastructural investigations were performed by transmission electron microscopy.
Results: Syntaxin 1A and SNAP25 was found to associate in clusters along the plasma membrane. The size of the clusters was estimated to be 323 ± 10nm ($n=48$) and each B-cell contained 425 ± 22 ($n=9$) SNAP25/syntaxin 1A clusters. After treatment with methyl-beta-cyclodextrin (mbcd) Western Blot analysis showed movement of SNAP25 from the plasma membrane to the cytosolic fraction. Insulin-secretion was reduced by 30% ($n=8$; $P<0.05$) after 30 min pre-treatment with 0.1 mM mbcd treatment. Inhibition was overcome by addition of cholesterol ($P<0.01$; $n=8$). Capacitance measurements revealed a ~50% inhibition of the exocytotic response and the total increase in membrane capacitance evoked by a train of ten depolarizations from -70 mV to 0 mV was reduced from 211 ± 38 fF ($n=6$) under control conditions to 96 ± 25 fF ($n=7$; $P<0.02$) after treatment with 0.1 mM mbcd. Treatment with mbcd was also associated with a 30% ($P<0.05$; $n=13–14$) reduction in the voltage-dependent peak Ca^{2+}-current. However, this reduction in peak current only account for part of the diminished exocytotic response. Electron microscopic analysis revealed that the number of docked granules in the B-cells was slightly reduced by 17% ($n=45–54$) in islets treated with 5 mM mbcd.
Conclusion: Our data indicate that 1) SNARE-proteins are arranged in clusters in primary B-cells. 2) A low concentration of the cholesterol-depleting compound mbcd is enough to reduce exocytosis and insulin-secretion. 3) We suggest that the formation of cholesterol-rich domains in the plasma-membrane, where SNARE-proteins cluster, are necessary for efficient fusion in pancreatic B-cells.

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Real-time imaging of the insulin response in single insulin-secreting cells

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Background and Aims: Insulin receptor-mediated activation of PI3-kinase with resulting formation of the lipid messenger phosphatidylinositol-3,4,5-trisphosphate (PIP_3) plays an important role in the regulation of a variety of β-cell functions, including gene transcription, cell proliferation, survival and insulin secretion. The present study aimed at investigating the dynamics of PIP_3 in individual insulin-secreting cells stimulated with insulin and various insulin secretagogues.
Materials and Methods: Insulin-secreting MIN6 cells were transfected with the PIP_3-binding pleckstrin homology domain from Akt tagged to GFP (AktPH-GFP). This construct resides in the cytoplasm under basal conditions but translocates to the plasma membrane upon generation of PIP_3. Fluorescence was recorded with confocal or evanescent wave microscopy. Cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured in parallel using the fluorescent Ca^{2+} indicator fura red.
Results: AktPH-GFP fluorescence was homogeneously distributed in the cytoplasm with a slight enrichment in the nucleus under basal conditions. Stimulation of the cells with 100 nM insulin resulted in rapid and sustained redistribution of the construct to the plasma membrane. This translocation appeared as an increase of evanescent wave excited AktPH-GFP fluorescence, reaching a maximum of 31 ± 3% above base-line within 63 ± 7 s. The insulin response was dose-dependent with half-maximal and maximal effects at ~560 pM and ~100 nM, respectively. The translocation was reversed upon removal of insulin and completely abolished by 100 μM of the PI3-kinase inhibitor LY294002. Simultaneous recording of AktPH-GFP membrane fluorescence and $[Ca^{2+}]_i$ during stimulation of insulin secretion with 30 mM KCl revealed that the depolarization-induced rise of $[Ca^{2+}]_i$ was followed by rapid formation of PIP_3 that was dependent on the presence of Ca^{2+} in the extracellular medium. Similarly, stimulation of insulin secretion by elevation of the glucose concentration from 3 to 20 mM resulted in a rise of $[Ca^{2+}]_i$ followed by pronounced formation of PIP_3 (30 ± 7% increase of fluorescence).
Conclusion: Insulin secretion from MIN6 β-cells is associated with rapid auto- and/or paracrine stimulation of PIP_3 generation in the plasma membrane. The presently used approach for single-cell monitoring of plasma membrane PIP_3 concentration provides an important tool for further studies of the role of this lipid messenger in the regulation of β-cell function.

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ELKS, a protein structurally related to the active zone associated protein CAST, is expressed in pancreatic beta cells and functions in insulin exocytosis

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Background and Aims: The cytomatrix at the active zone (CAZ) has been implicated in defining the site of Ca2+-dependent exocytosis of neurotransmitters. In order to clarify the functions of the CAZ protein in insulin exocytosis, we examined the expression and function of ELKS, a protein structurally related to the CAZ protein CAST in insulin exocytosis from pancreatic b cells.
Materials and Methods: Rat pancreas and MIN6 b cells were immunostained with anti-ELKS, anti-syntaxin 1 and anti-insulin antibodies and then observed by electron, confocal and total internal reflection fluorescence (TIRF) microscopies. The interaction between the docking/fusion of GFP-tagged insulin granules and ELKS labeled by TAT-conjugated Cy3-tagged monoclonal antibody was analyzed by dual color TIRF microscopy using live MIN6 cells.
Results: The results of confocal and immunoelectron microscopic analysis showed that ELKS is present in pancreatic b cells and is localized close to insulin granule docked on the plasma membrane-facing blood vessels. TIRF microscopy imaging in MIN6 cells revealed that the ELKS clusters are less dense and unevenly distributed than syntaxin 1 clusters, which are enriched in the plasma membrane. Most of the ELKS clusters were on the docking sites of insulin granules that were colocalized with syntaxin 1 clusters. TIRF images of single granule motion showed that the fusion events of insulin granules were mostly occurred on the ELKS cluster, where repeated fusion was sometime observed. Immunoprecipitation study showed that ELKS formed a ternary complex with the other CAZ proteins, Bassoon and RIM2. When the Bassoon-binding region of ELKS was introduced into MIN6 cells, the docking/fusion of insulin granules were markedly reduced, suggesting that ELKS together with Bassoon is involved in insulin exocytosis. The attenuation of ELKS expression by siRNA decreased the number of ELKS clusters in the plasma membrane and reduced the glucose-evoked insulin release.
Conclusion: ELKS is a possible candidate to define the fusion site of insulin exocytosis, and it regulates docking and fusion of insulin granules. Thus, the function of CAZ-related protein is not only limited to formation of the CAZ structure in neuron but also may be applicable to insulin exocytosis.

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A novel regulated exocytotic pathway mediates glucose-induced K_{ATP} channel recruitment to the β-cell plasma membrane

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Background and aims: Dogmatically, neurons, endocrine and exocrine cells are believed to use a regulated exocytotic pathway to output secretory molecules, such as neurotransmitters, hormones and enzymes, and a constitutive pathway to renew its integral membrane proteins and lipids. On the contrary, other types of cells are thought to possess only the constitutive pathway and thus called constitutive cells. However, experimental evidence demonstrates that the so-called constitutive cells are also equipped with the regulated exocytotic pathway to deliver integral membrane proteins from *trans*-Golgi network to the plasma membrane. The β-cell as a highly specialized secretory cell uses stimulated exocytosis to serve its primary function insulin secretion. As with constitutive cells, a constitutive secetory pathway also operates in the β-cell to renew its integral membrane proteins and lipids. However, it is not know if the β-cell is equipped with a resemblance to the constitutive cell-equipped regulated exocytotic pathways. This work aims to identify the pathway and mechanism responsible for glucose-induced K_{ATP} channel translocation to the β-cell plasma membrane.
Material and methods: Experiments were performed in mouse β-cells using patch-clamp recording, subcellular fractionation and immunoblot analysis and confocal microscopy.
Results: Whole-cell and unitary K_{ATP} current recordings show that stimulation with 17 mM glucose for 1 h led to a significant increase in whole-cell K_{ATP} currents without influencing single K_{ATP} channel property in β-cells, dialyzed with 0.3 mM ATP/ADP and exposed to the K_{ATP} channel opener diazoxide. Subcellular fractionation and immunoblot analysis reveal that the glucose stimulation also caused a marked shift of Kir6.2 and SUR1 subunits from intracellular compartments to the plasma membrane. The effect was partially blocked by the PKA inhibitor H-89. The glucose stimulation did not alter abundance of total protein, Kir6.2 and SUR1 subunits. Importantly, immunofluorescence labeling and confocal microscopy demonstrate that anti-SUR1 and anti-Kir6.2 antibodies recognized abundant granule-like structures in the cytoplasm. These structures lacked insulin immunoreactivity. This rules out the possibility that β-cells use the insulin exocytotic pathway to transport SUR1 or Kir6.2 subunits to the plasma membrane. Moreover, these abundant structures with the SUR1 and Kir6.2 subunits are unlikely to be classical constitutive vesicles since the constitutive vesicles bud off from *trans*-Golgi network and immediately go to the plasma membrane in an unregulated manner. Therefore, we refer to this novel pathway as the regulated non-insulin exocytotic pathway.
Conclusion: Our data demonstrate that a novel regulated exocytosis, i. e. the regulated non-insulin exocytosis, mediates the glucose-induced K_{ATP} channel recruitment to the β-cell plasma membrane, partially dependent on PKA phosphorylation. This novel mechanism regulates the number of functional K_{ATP} channels in the β-cell plasma membrane. It endows glucose with an additional function in the β-cell, namely to facilitate functional protein translocation by stimulating regulated non-insulin exocytosis. Such a mechanism might function as a β-cell feedbacker to allow the cell to quickly return to resting membrane potential after glucose stimulation and be of vital importance in guaranteeing the characteristic β-cell phenotype.

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The control of insulin secretion by islets from 2 week-old sulfonylurea receptor 1 knockout mice

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Background and Aims: Sulfonylurea receptor 1 knockout (Sur1KO) mice lack functional K_{ATP} channels in β-cells. In vitro studies of their islets at an adult age have yielded conflicting results. Insulin secretion (IS) was found to be low in the basal state and barely stimulated by high glucose (G). In other studies, basal IS was elevated and G stimulated IS virtually without changing the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$), thus via an amplifying (K_{ATP} channel-independent) pathway. Sur1KO mice were developed originally as a model for congenital hyperinsulinemic hypoglycemia, but the physiology of their islets at a young age has not been investigated. This was the aim of the present work.
Materials and Methods: Islets were isolated from control and Sur1KO mice at 12–14 days of age. After 18 h culture in RPMI medium containing 10 or 5 mmol/l G (G10 or G5), islets were perifused for measurement of IS or $[Ca^{2+}]_i$ (fura PE3). The Krebs medium was supplemented with 1 μmol/l forskolin to increase β-cell cAMP.
Results: Body weight (6.7 ± 0.1 vs 5.6 ± 0.1 g, SEM), blood glucose (5.7 ± 0.1 vs 4.9 ± 0.1 mmol/l) and islet insulin content (35 ± 1 vs 30 ± 1 ng/islet) were higher ($P<0.001$) in Sur1KO vs control mice. Unlike control islets in which diazoxide decreased and tolbutamide increased both $[Ca^{2+}]_i$ and IS, respectively, Sur1KO islets were insensitive to the drugs, attesting to the absence of functional K_{ATP} channels. In control islets, increasing glucose from G1 to G15 induced typical biphasic increases in $[Ca^{2+}]_i$ and IS. Tolbutamide, or high KCl + diazoxide, increased $[Ca^{2+}]_i$ and IS in G1. Under these conditions, G15 augmented IS further without increasing $[Ca^{2+}]_i$. After overnight culture in G10, Sur1KO islets perifused with G1 showed elevation of both $[Ca^{2+}]_i$ and IS. Upon stimulation with G15, $[Ca^{2+}]_i$ increased transiently while IS augmented progressively but steadily (6-fold). This effect of glucose via the amplifying pathway persisted in the presence of tolbutamide (inactive) or KCl + diazoxide. Overnight culture of control islets in G5 instead of G10 decreased the magnitude of the changes in $[Ca^{2+}]_i$ and IS without affecting their time course. The impact of culturing Sur1KO islets in G5 was spectacular. During perifusion with G1, $[Ca^{2+}]_i$ and IS were no longer elevated but similar to those in controls. Most unexpectedly for β-cells without K_{ATP} channels, G15 triggered biphasic increases in both $[Ca^{2+}]_i$ and IS, albeit smaller than in controls. These biphasic responses to glucose persisted in the presence of tolbutamide, but not after depolarization with KCl.
Conclusion: Glucose control of IS through the amplifying pathway is already operative in β-cells of 2 week-old normal mice. The lack of K_{ATP} channels in Sur1KO β-cells causes an inappropriate elevation of $[Ca^{2+}]_i$ and IS in low glucose, but does not prevent high glucose from increasing IS via the amplifying pathway. Overnight culture of Sur1KO islets in G5 suffices to mask the intrinsic abnormalities of $[Ca^{2+}]_i$ and IS observed in low glucose

after culture in G10. $[Ca^{2+}]_i$ homeostasis thus remains under metabolic control in these β-cells without K_{ATP} channels. Since the blood glucose of these young mice is closer to 5 than 10 mmol/l glucose, inappropriate elevation of basal $[Ca^{2+}]_i$ and IS may be chronically prevented, explaining, in part, why the mice are not hypoglycemic.

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Pancreatic development and monogenic diabetes

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Neonatal diabetes mellitus: understanding the molecular basis of human pancreas development

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Background and Aims: Severe and permanent neonatal diabetes mellitus in humans is associated with pancreatic agenesis /hypoplasia. The underlying pathogenesis is unknown but inherited mutations in genes that control pancreatic development have been implicated in some instances. Recently, considerable progress has been made in identifying genes that control pancreatic morphogenesis and development in experimental animals using gene deletion approaches. Most of these genes are pancreatic transcription factors, such as Sox17, Hlxb9, Hnf-6, Ptf1a, Pdx1, Isl-1, Hnf3β, Pbx-1, Hes1 and Ngn3. Pdx-1 knockout mice, for example, fail to develop a pancreas and mutations in the Pdx1 promoter E-box region have been shown to abolish PDX1 protein expression in cell culture. Much less is known about the control of pancreas development in humans but, there are cases of human pancreatic agenesis that have been traced to homozygous Pdx1 mutations, a single mutant allele having been inherited from each unaffected, heterozygous parent. We have identified four patients with permanent neonatal diabetes mellitus who lack detectable pancreas. All come from consanguineous families and two come from the same family indicating that a recessive genetic defect may be responsible for the disease. The aim of this study was to analyse the genes encoding pancreatic transcription factors in these probands to determine whether their pancreatic agenesis could be attributed to Pdx-1 mutations or mutations in other pancreatic transcription factors.
Materials and Methods: Blood samples were obtained (with consent and ethical approval) from the subjects and genomic DNA was extracted using standard procedures. A number of candidate genes known to be important in pancreas development in the mouse were amplified by PCR and sequenced using the dye-labelled dideoxy chain termination method. To attempt to identify the mutation causal for the pancreatic agenesis we analysed the coding regions of Pdx-1, Sox17, Hlxb9, Hnf-6, Ptf1a, and also the promoter region (E-box) of the Pdx-1 gene.
Results: In contrast to previous reports in pancreatic agenic humans, we were unable to attribute the pathology to a mutation in the coding sequence of a known transcription factor. Thus, no mutations were found in the coding sequence of Pdx-1, Sox17, Hlxb9, Hnf-6 and Ptf1a. In addition, we were unable to detect any abnormalities of splicing sequence in intron-exon boundaries in these genes in the 4 subjects. Interestingly, a sequence variant in the promoter region (E-box) of the Pdx-1 gene (176G>A, upstream of transcription start site) in all four subjects was identified but the importance of this is unclear.
Conclusion: Studies in knockout mice have identified a number of candidate genes that are important for pancreas development. Our study in consanguineous probands with severe neonatal diabetes and pancreatic agenesis suggests that mutations in other genes may be responsible for pancreatic agenesis in humans, and imply that the regulation of pancreatic development in humans may differ substantially from that in mice. Further studies of this consanguineous cohort, including homozygosity mapping, should yield useful information about the unidentified genes involved in pancreas development in humans.

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Mutations in the hepatocyte nuclear factor-1beta gene cause neonatal diabetes and intra-uterine growth retardation: support for a critical role of HNF-1beta in human pancreatic development

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Background and Aims: The transcription factor hepatocyte nuclear factor-1beta (HNF-1beta) was recently detected in rodent pancreatic progenitor

cells. Its role in human beta-cell development is uncertain, although an *HNF-1β* mutation (S148W) was recently described in a patient with neonatal diabetes. Our study aims were to determine if mutations in *HNF-1β* are a common cause of neonatal diabetes and if *HNF-1β* mutation carriers had an altered birth weight.
Materials and Methods: To investigate the role of HNF-1beta in early beta-cell development we examined birth weight (a reflection of insulin mediated growth) in 20 patients with *HNF-1β* mutations. We also sequenced the *HNF-1β* gene in 21 patients with neonatal diabetes in whom other known genetic aetiologies had been excluded.
Results: Birth weight was markedly reduced [median(range) 2.4(1.83–3.3) kg]. A novel heterozygous *HNF-1*β mutation, S148L, was identified in one patient who was small for gestational age (1.83 kg). He was diagnosed at 17 days with diabetes that resolved but relapsed at 8 years, requiring insulin treatment again. There is evidence of exocrine insufficiency.
Conclusion: The low birth weight associated with *HNF-1β* mutations is consistent with reduced insulin secretion in utero. We confirm that *HNF-1β* mutations can result in resolving neonatal diabetes, especially when the S148 residue is mutated. We conclude that HNF-1beta plays a critical role in early human pancreatic development altering both pancreatic endocrine and exocrine function. This suggests a key role for HNF-1beta in early pancreatic progenitor cells.
Support: South west multi-centre research ethics committee

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Subjects with HNF-1 beta mutations have isolated insulin resistance of endogenous glucose production rate in contrast to normal insulin sensitivity in subjects with HNF-1 alpha mutations
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Background and Aims: Heterozygous mutations in Hepatocyte Nuclear Factor-1 (HNF-1) alpha and HNF-1 beta cause maturity onset diabetes of the young (MODY) type 3 and type 5 respectively. Despite structural similarity between HNF-1 alpha and beta, subjects with HNF-1 beta mutations have hyperinsulinaemia. We hypothesised that subjects with HNF-1 beta mutations would have insulin resistance compared to subjects with HNF-1 alpha mutations and due to the pattern of tissue expression this would be hepatic and/or renal insulin resistance
Materials and Methods: We performed a 2 step (0.3 mU/kg/min and 1.5 mU/kg/min) hyperinsulinaemic euglycaemic clamp with infusion of [6,6-$^2H_2$]glucose in 6 subjects with HNF-1 alpha mutations (mean±SEM age 35.5 ± 4.63 years, BMI 27.12 ± 1.07 kg/m^2), 6 subjects with HNF-1 beta mutations (age 34.5 ± 4.05 years, BMI 26.17 ± 1.13 kg/m^2) and 6 healthy controls (age 34.33 ± 4.55 years, BMI 25.97 ± 1.30 kg/m^2). Glucose concentration was maintained using dextrose spiked with [6,6-$^2H_2$]glucose. Isotopic enrichment of glucose was measured by gas chromatography-mass spectrometry. Non-esterified fatty acids were measured to provide an estimate of lipolysis
Results: Low dose (LD) insulin suppressed endogenous glucose production (Ra) by greater than 75% in subjects with HNF-1 alpha mutations (10.85 ± 1.20 v 1.09 ± 0.85 μmol/kg/min. Basal v LD; p = 0.001) and control subjects (9.27 ± 0.43 v 1.81 ± 0.61 μmol/kg/min; p < 0.001) but had no significant effect on endogenous glucose Ra in subjects with HNF-1 beta mutations (9.49 ± 1.65 v 7.11 ± 1.64 μmol/kg/min; p=0.23). All groups showed full suppression of endogenous glucose Ra with high dose (HD) insulin and there was no significant difference in insulin stimulated glucose uptake or suppression of lipolysis between the 3 groups with LD or HD insulin
Conclusion: Subjects with HNF-1 beta mutations have isolated resistance of endogenous glucose production to insulin with normal peripheral insulin sensitivity. In contrast subjects with HNF-1 alpha mutations have normal insulin sensitivity. HNF-1 is a mediator of the effect of insulin on glucose-6-phosphatase, a key gluconeogenic enzyme.This unique finding suggests that there may be differential regulation of glucose-6-phosphatase by HNF-1 alpha and HNF-1 beta.
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The extent of K_{ATP} channel block by MgATP correlates with the clinical phenotype caused by gain-of-function *KCNJ11* mutations
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Background and Aims: ATP-sensitive K^+ (K_{ATP}) channels control electrical signalling in diverse cell types by coupling metabolism to transmembrane K^+ fluxes. They comprise pore-forming Kir6.2 and regulatory sulphonylurea receptor (SUR) subunits. Metabolic regulation is mediated by changes in intracellular adenine nucleotides: ATP binding to Kir6.2 inhibits, and interaction of MgATP with SUR, increases, channel activity. In pancreatic beta-cells, K_{ATP} channels mediate glucose-stimulated insulin secretion. Heterozygous (*het*) mutations in Kir6.2 (*KCNJ11*) cause permanent neonatal diabetes alone (PNDM; R201H/C) or in association with developmental delay, muscle weakness, and epilepsy (DEND syndrome; Q52R, V59G, I296L). Functional analysis in the absence of Mg^{2+}, to isolate the effects of ATP on Kir6.2, showed both types of mutation reduce channel inhibition by ATP. However, in cells, K_{ATP} channel activity is governed by the balance between ATP inhibition via Kir6.2 and Mg^{2+}-nucleotide stimulation mediated by SUR. We therefore studied the MgATP sensitivity of Kir6.2 mutant channels.
Materials and Methods: K_{ATP} currents were recorded from *Xenopus laevis* oocytes injected with wild-type (*wt*) or mutant Kir6.2 and SUR1 mRNA. To simulate the *het* state, we used a 1:1 mixture of *wt* and mutant Kir6.2 mRNAs. Macroscopic (or single-channel) currents were recorded by patch-clamping inside-out patches. The pipette solution contained (mM): 140 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$, 10 HEPES (pH 7.4). The Mg^{2+} free internal (bath) solution contained (mM): 107 KCl, 1 K_2SO_4, 10 EGTA, 10 HEPES (pH 7.2). The Mg^{2+}-containing internal solution consisted of Mg^{2+}-free solution plus 2 mM $MgCl_2$ and MgATP (instead of ATP).
Results: Mg^{2+} caused a small increase (1.9 ± 0.4-fold; n=6) in the IC_{50} (13 ± 1 μM) for ATP inhibition of *wt* channels. In contrast, the IC_{50} for homozygous R201C and R201H mutant channels was dramatically increased: IC_{50} = 2.4 ± 0.2 mM (n=5) and 2.0 ± 0.2 mM (n=6) (6.5 ± 0.8-fold and 23.5 ± 2.9-fold increase) respectively. Mg^{2+} dramatically altered the shape of the dose-response curve of homozygous Q52R, V59G and I296L mutant channels, resulting in a substantial fraction (35–87%) of unblocked current at high MgATP. The fraction of unblocked current in 3 mM MgATP for het channels was correlated with disease severity, with V59G(40%) > I296L(32%) > Q52R(27%) > R201C(15%) > R201H(8%).
Conclusion: Taken together, these results support the idea that neonatal diabetes results from an increased K_{ATP} current in beta-cells, which reduces electrical activity and insulin secretion. Larger increases in K_{ATP} current may be required to influence electrical activity in other cells types and cause the symptoms associated with DEND syndrome.

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Identification of a new mutation in the *KCNJ11* gene in a patient affected by severe congenital hyperinsulinism (CHI)
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Background and Aims: Congenital hyperinsulinism (CHI), a disease presenting with severe hypoglycemia in the newborn period, can be caused by mutations in the pancreatic ATP-sensitive potassium channel. The K_{ATP} channel, localized at the beta cell membrane, consists of two subunits, the sulfonylurea receptor 1 (*SUR1*) and the inwardly rectifying potassium channel *Kir6.2*. We have identified a new homozygous missense mutation in the *KCNJ11* gene, coding for the Kir6.2 subunit in a patient severely affected by the disease.
Materials and Methods: The Kir6.2 $_{H259R}$ mutation was generated in vitro by site-directed mutagenesis in the cDNA of the human *KCNJ11* gene. Kir6.2 $_{WT}$ or Kir6.2 $_{H259R}$ was co-expressed with SUR1 in HEK293T cells. Currents of wild type and mutant K_{ATP} channels were studied using the patch clamp technique in the whole cell configuration. Double immunoflu-

orescence and advanced life fluorescence imaging, were used to analyze the localization of the Kir6.2 protein.
Results: The patient was born at term and severe hypoglycemic episodes were observed 30 minutes after birth. Continuous iv glucagon (1 mg/d) was needed to stabilize the blood glucose levels. Pancreatic catheterization with measurements of insulin levels revealed a diffuse form of CHI and a subtotal pancreatectomy (97%) was performed. Genetic analysis revealed a new mutation (H259R) in the *KCNJ11* gene. Monitoring K_{ATP} current in revealed a complete loss in mutant versus the wild type channel ($Kir6.2_{WT}$: Gm =2.40 ± 1.38 nS/pF, n = 9 versus $Kir6.2_{H259R}$ Gm = 0.017 ± 0.018 nS/pF, n = 8, $p < 0.01$). We tested the hypothesis that the loss of K_{ATP} current was caused by an impaired trafficking of the K_{ATP} channel to the cellular membrane. While there was significant co-localization of the Kir6.2 protein with markers of endoplasmatic reticulum ($Kir6.2_{WT}$, 0.21%± 0.16%, n = 5 versus $Kir6.2_{H259R}$ 3.44%± 1.09%, n=5. $p < 0.05$, t-test), this was not the case for markers of the Golgi apparatus ($Kir6.2_{WT}$ 1.28%± 0.09%, n = 4, $Kir6.2_{H259R}$ 1.67%± 0.28%, n = 5. NS, t-test). Finally, these results were confirmed by total internal reflection fluorescence (TIRF) showing a marked decrease in expression of the mutant channel at the cellular membrane.
Conclusion: By combining molecular biology, cellular physiology and cellular imaging, we have determined that the newly identified $Kir6.2_{H259R}$ mutation leads to a partial retention in the ER and to a non-functional channel when expressed at the cellular membrane. Therefore, the amino acid H259 seems to play an important role in the generation of a functional K_{ATP} channel at the beta-cell membrane.
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Association of the E23K polymorphism in the Kir6.2 (*KCNJ11*) gene with gestational diabetes mellitus
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Background and Aims: Gestational diabetes mellitus (GDM) and type 2 diabetes share common pathophysiological background such as β-cell dysfunction and insulin resistance. In addition, women with GDM are at high risk of developing type 2 diabetes later in life. Our aim was to investigate whether GDM has a similar genetic predisposition as type 2 diabetes by studying 5 common polymorphisms in 4 candidate genes that have been previously associated with type 2 diabetes.
Materials and Methods: We studied 1777 unrelated Scandinavian women (588 GDM and 1189 pregnant non-diabetic controls) for the pancreatic β-cell ATP-sensitive K^+ (K_{ATP}) channel subunit (Kir6.2 E23K), insulin receptor substrate-1 (*IRS-1* G972R), uncoupling protein 2 (*UCP2* -866G/A), and Calpain-10 (*CAPN10* SNP43 & -SNP44) polymorphisms.
Results: The genotype frequencies of the Kir6.2 E23K polymorphism differed significantly between GDM and matched control women (31.5%, 52.7%, 15.8% vs. 37.3%, 48.8%, 13.9% for EE, EK and KK genotypes respectively, p=0.050). Also, the K-allele was more common in GDM women compared to controls (42.2% vs. 38.3%, p=0.026). In addition, the K-allele was associated with GDM under a dominant model (KK/EK vs. EE) (68.5% vs. 62.7%, p=0.015). Analysis of the *IRS-1* G972R polymorphism showed that RR homozygosity was found exclusively in GDM women (91.0%, 8.3%, 0.7% vs. 90.7%, 9.3%, 0.0% for GG, GR and RR genotypes respectively, p=0.014) and this was statistically significant under a recessive model (RR vs. GR/GG) (0.7% vs. 0.0%, p=0.011). The genotype and allele frequencies of the other studied polymorphisms did not statistically differ between GDM and control women.
Conclusion: The E23K polymorphism of the *KCNJ11* gene predisposes to GDM in Scandinavian women.

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New avenues for treatment of nephropathy

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Effect of raloxifene on urinary albumin excretion in post-menopausal type 2 diabetic women: a randomized, placebo-controlled trial
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Background and Aims: Diabetic nephropathy (DN) is the leading cause of end stage renal disease in France. Women have a lower risk for DN than men; the determinants of this protective effect are still poorly understood. We tested the hypothesis of a protective effect of raloxifene on urinary albumin excretion in type 2 diabetic post-menopausal women in a randomised clinical trial.
Materials and Methods: 39 post-menopausal type 2 diabetic women (aged 66 +/– 7 years) with micro- or macro-albuminuria on top of recommended therapy for DN entered a double-blind placebo controlled trial for a duration of 6 months: 20 were randomised to placebo and 19 to raloxifene 60 mg/day. First morning urinary albumin/creatinine ratio (ACR) was determined on 3 consecutive days in the week before randomisation and before the final visit.
Results: One patient in each group discontinued in the first 3 weeks, leaving 37 patients for intent-to-treat analysis (19 on placebo and 18 on raloxifene). In the placebo group, mean ACR increased between randomisation and final visit from 277 mg/g (67–651) [median (interquartile range)] to 284 mg/g (79–1508) (p=0.0305) while it slightly decreased in the raloxifene group from 376 mg/g (67–615) to 243 mg/g (103–549). Those patients on placebo had a change in ACR between randomisation and final visit of +24 (–37;+517) vs –10 mg/g (–36;+16) in the raloxifene group (p=0.0246). In multivariate analysis accounting for baseline ACR, blood pressure, lipids or HbA1c, only raloxifene treatment explained the change in ACR. Adverse events were not differently encountered in both groups.
Conclusions: These results support the hypothesis that raloxifene reduces albuminuria in postmenopausal women with diabetes; further study in a larger population is warranted.

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Effect of ruboxistaurin on albuminuria and estimated glomerular filtration rate (GFR) in persons with type 2 diabetes and nephropathy
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Background and Aims: In animal models, diabetes-induced renal disease was ameliorated by ruboxistaurin (RBX), a selective inhibitor of protein kinase C β. A randomized, double-blind, placebo-controlled, multicenter, pilot phase 2 trial was conducted to determine whether RBX 32 mg/d for 1 year could lower the urinary albumin/creatinine ratio (ACR) in persons (n=123) with type 2 diabetes, and albuminuria (ACR 200–2000 mg/g) despite stable doses of angiotensin converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs) or both.
Materials and Methods: 123 patients were randomized to either placebo or RBX. All patients were on stable doses of ACEIs or ARBs or both for 6 months prior to the study and these drugs were continued for the entire trial. Urinary albumin and creatinine were measured at baseline, 1, 3, 6 and 12 months. GFR was estimated (eGFR) using the modified MDRD formula at baseline and endpoint. All data were analyzed using an intention-to-treat principle. Missing values were defined by last-observation-carried-forward. Dichotomous variables were analyzed using Fisher's exact test and continuous variables were analyzed using analysis of variance (ANOVA) based on rank-transformed data. Other statistical analyses were performed using an Analysis of Covariance (ANCOVA) model.

Results: At baseline, overall urinary ACR was 764 ± 430 mg/g (mean±SD), and eGFR was 70 ± 24 ml/min per 1.73 m^2. Baseline characteristics did not differ significantly between treatment groups. At endpoint, ACR decreased significantly from baseline after one year of treatment (–24%, p=0.02) with RBX 32 mg/day. ACR did not change significantly from baseline in the placebo group (–9%, p=0.33). In the RBX 32 mg/d group, the ACR-lowering effect of RBX appeared as early as 1 month, was maximal at 3 months and was sustained for the 1-year period of the study. In the placebo group, participants lost significant eGFR over 1 year (–4.8 ± 1.8 ml/min/year, p=0.009). In contrast, the RBX group had no significant change in eGFR (–2.5 ± 1.9 ml/min/year, p=0.185). When treatment groups were compared, changes in ACR and eGFR were not statistically significant. However, this pilot phase 2 study was not designed to determine such a difference and the power for either analysis was less than 20%. Treatment-emergent adverse events were similar between groups.
Conclusion: The addition of RBX to ACEIs or ARBs, or both, for one year, lowered ACR and was not associated with a significant reduction in eGFR in this pilot study of participants with type 2 diabetes and nephropathy. RBX may be a useful addition to established therapies for diabetic nephropathy.

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Prevention of glomerular macrophages infiltration and renal MCP-1 expression by rosiglitazone in experimental diabetes

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Background and Aims: Whether insulin-sensitizers have metabolically and/or haemodynamically independent direct renoprotective effects is a matter of debate. Macrophage infiltration within the glomeruli has been demonstrated in patients with diabetic nephropathy and inflammatory mechanisms may contribute to the development of the glomerular injury in diabetes. MCP-1, a potent monocyte chemoattractant, is over-expressed in the glomeruli in experimental diabetes. In this study we tested the effects of Rosiglitazone (R), a PPAR-γ agonist and potent insulin sensitizer, on the development of macrophage infiltration and monocyte chemoattractant protein-1 (MCP-1) expression, in streptozotocin-induced diabetic Sprague-Dawley rats.
Materials and Methods: Four groups of 6–8 Sprague Dawley rats of 150–200 g body weight (bw), were studied: diabetic rats (D), diabetic rats treated with R (DR), non-diabetic rats (C), non-diabetic rats treated with R (CR). Diabetes was induced by a single intraperitoneal injection of streptozotocin 65 mg/kg bw and confirmed 48 h later by a blood glucose level higher than 22 mM. Diabetic animals were treated daily with subcutaneous long acting insulin, 0.005–0.01 U/g bw, to maintain glycaemia at around 24–26 mM. R was administered as a powder mixed in the food at a dose of 0.005 mg/g bw/day after adjustment for animal weight and food consumption. D and DR animals were pair fed. Body weight and blood glucose (one Touch glucose-meter) were measured weekly. Blood pressure (tail cuff non-invasive method) was monitored monthly throughout the study. Animals were sacrificed at 1, 3, and 9 months. Glomerular macrophage infiltration was measured with specific macrophage marker (ED1) on kidney frozen sections (one section every 150 microns, 50 glomeruli per animal) within the intraglomerular (glomerular boundaries: external perimeter capillary loops) and extraglomerular space (within a surface area of 1 mm^2), while MCP-1 expression was determined by western immunoblotting techniques on total kidney lysate.
Results: Rat body weight was lower at 9 months in D (mean±sem, 458 ± 32.9 g) versus C (565 ± 53 g) but did not differ between D and DR (471 ± 3 g). Blood glucose was elevated in the diabetic rats, stable throughout the study, and did not differ between D (30 ± 5.2 mM) and DR (29 ± 3.5 mM). Blood pressure was not statistically different in D (systolic 165 ± 7, diastolic 73 ± 10 mmHg) *vs* DR (152 ± 8, 86 ± 6) and in C (167 ± 6, 84 ± 8) *vs* CR (157 ± 9, 79 ± 7). Analysis of macrophage infiltration showed an intraglomerular macrophage accumulation (3 months) followed by a more pronounced intra and extraglomerular cortical infiltrate (9 months) in D; the macrophage infiltrate observed in D was completely prevented by R at both 3 and 9 months. No macrophage infiltration was observed in C and CR. Similarly MCP-1 expression at 9 months was upregulated in D and this was prevented by R to levels similar to controls (C and CR) (p<0.03).
Conclusion: Rosiglitazone prevents glomerular macrophage infiltration and renal MCP-1 expression in streptozotocin-diabetic rats. These effects are independent of blood glucose and blood pressure levels control. Modulation of immunological/inflammatory response by R may be important for the PPAR-γ ligand renoprotective action.
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Protective effects of thiazolidinedione against renal injuries are mediated by anti-inflammatory actions through PPAR-γ in experimental diabetic rats

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Background and Aims: Thiazolidinedione (TZD), a ligand for peroxisome proliferator-activated receptor-γ (PPAR-γ), is known to show anti-inflammatory actions independent of blood glucose lowering effects. TZDs show beneficial effects on atherosclerosis through anti-inflammatory actions. Recent studies have suggested that inflammatory proscess is also involved in the pathogenesis of diabetic nephropathy. We have recently reported that TZD ameliorates renal injuries through anti-inflammatory actions in experimental diabetic rats (Diabetologia 47 Suppl.1 A398 2004, Abstr.). Pioglitazone reduced albuminuria and glomerular hypertrophy in streptozotocin-induced diabetic rats without change of blood glucose levels. Pioglitazone also reduced expression of ICAM-1, infiltration of macrophages, expression of TGF-β and type IV collagen in the kidney of diabetic rats. The aim of this study is to further evaluate the intracellular molecular mechanisms of anti-inflammatory effects of TZD on diabetic nephropathy.
Materials and Methods: We divided Sprague-Dawley rats into three groups: (i) non-diabetic control rats (non-DM); (ii) diabetic rats (DM) and (iii) diabetic rats treated with pioglitazone (DM+pio). The DM+pio group received 0.0002% pioglitazone mixed in chow. The kidneys were harvested at 8 weeks after the induction of diabetes. In addition to the inflammatory parameters, we analyzed the activation for nuclear factor-kappa B (NF-κB) in renal tissues using electrophoretic mobility shift assay (EMSA). We examined the distribution of PPAR-γ in the kidney by immunostaining using anti-PPAR-γ antibody and anti-endothelial cell antibody (RECA). Gene expression of PPAR-γ mRNA was investigated in both cultured human glomerular endothelial cells (GENC) and human microvascular endothelial cells (HMVEC). GENC and HMVEC were incubated in different glucose concentrations (5.5 or 30 mM) with or without pioglitazone, ciglitazone (PPAR-γ agonist) and pyrrolidine dithiocarbamate (PDTC; NF-κB inhibitor) for 24 h. We evaluated the protein expression of ICAM-1 by Western blot analyses and analyzed the activities of NF-κB by EMSA.
Results: Renal NF-κB activity was increased in diabetic rats and reduced by pioglitazone. Immunohistochemistry revealed that PPAR-γ protein was distributed in both glomeruli and tubuli. Immunostaining in serial tissue sections revealed that glomerular endothelial cells (RECA-1 positive cells in glomeruli) express PPAR-γ. High glucose condition activated NF-κB and increased the expression of ICAM-1 in GENC and HMVEC. Pioglitazone,ciglitazone and PDTC reduced the activities of NF-κB and expression of ICAM-1 in these endothelial cells cultured under high glucose condition.
Conclusion: 1) Pioglitazine prevents the activation of NF-κB and expression of ICAM-1 resulted in decrease of macrophage infiltration in diabetic kidneys. 2) Glomerular endothelial cells express PPAR-γ. 3) NF-κB activities and ICAM-1 expression are increased under high glucose condition and reduced by TZD in cultured endothelial cells. We conclude that the beneficial effects of pioglitazone for diabetic nephropathy might be mediated by its anti-inflammatory actions through PPAR-γ.

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Rosiglitazone improves glomerular hyperfiltration, renal endothelial dysfunction and microalbuminuria of incipient diabetic nephropathy in patients

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Background and Aims: Microalbuminuria (MA) an early feature of diabetic nephropathy indicates intrarenal endothelial damage. In NIDDM MA is strongly related to insulin resistance. We therefore investigated whether rosiglitazone - an insulin sensitising drug which is known to improve endothelial dysfunction - was able to improve intrarenal endothelial dysfunction and MA.
Materials and Methods: Nineteen NIDDM patients participated in this double-blind, cross-over trial. Nine of them with newly diagnosed disease without MA were randomised to a treatment with rosiglitazone or nateglinide, each for 12 weeks. Ten patients with MA were randomised to rosiglitazone or placebo, each for 12 weeks in addition to their previous

antidiabetic medication. After each treatment glomerular filtration rate (GFR), renal plasma flow (RPF) and filtration fraction (FF) were measured before and after blockade of NO by iv L-NMMA. Ten healthy subjects served as controls.
Results: NIDDM patients at baseline showed glomerular hyperfiltration compared to healthy controls. Rosiglitazone reduced elevated GFR and FF towards control primarily in patients with MA (GFR: 133.4 ± 9.8 vs. 119.6 ± 8.7 mL/min; FF: 23.2 ± 1.7 vs. 20.5 ± 1.6% without and with rosiglitazone, respectively ($p<0.05$ for each comparison); controls: GFR 111.7 ± 8.6 mL/min, FF 20.4 ± 1.5%). RPF was not significantly different between patients and controls and was not affected by treatment. Rosiglitazone improved intrarenal NO-bioavailability in NIDDM towards control as shown by infusion of L-NMMA (see table). Rosiglitazone reduced albumin excretion in NIDDM with MA from 116.5 ± 31 to 40.4 ± 12 mg/day. This reduction was closely related to the reduction of GFR ($r=0.9$, $p<0.01$).
Conclusion: Rosiglitazone ameliorated glomerular hyperfiltration in early NIDDM, improved NO bio-availability and lessened renal end organ damage in NIDDM with microalbuminuria.
Effects of L-NMMA on renal function (% change from baseline)

	controls	without MA		with MA	
		nateglinide	rosiglitazone	placebo + previous OAD	rosiglitazone + previous OAD
GFR	3.8 ± 5.9	−4.7 ± 5.4	2.1 ± 4.4	−3.6 ± 3.4	−2.3 ± 3.1
RPF	−10.9 ± 2.5*	−4.1 ± 3.4*	−13.1 ± 4.3*	−2.8 ± 6.6	−9.6 ± 3.1*
FF	17.4 ± 4.2*	−1.1 ± 2.5	16.5 ± 5.9*	−0.8 ± 3.1	11.9 ± 3.8*

*$p<0.01$ vs baseline; $p<0.02$ vs rosiglitazone
OAD: oral antidiabetic treatment

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Beneficial effects of thiazolidinediones on diabetic nephropathy
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Background and Aims: Diabetic nephropathy is a common complication in diabetes mellitus. Thiazolidinedione (TZD) is thought to ameliorate diabetic nephropathy, however, the mechanism has not been elucidated. We hypothesized that vascular endothelial growth factor (VEGF) and adiponectin participate in the pathogenesis of diabetic nephropathy and that TZD may be beneficial for the treatment of diabetic nephropathy through its effect on VEGF and adiponectin.
Materials and Methods: Twenty-three Otsuka-Long-Evans-Tokushima-Fatty (OLETF) rats were divided into three groups and 8 Long-Evans-Tokushima-Fatty (LETO) used as control group; control LETO (N=8), control OLETF (n=8), pioglitazone group (AD4833, 10 mg/kg/day in drinking water, n=7), rosiglitazone group (Avandia, 3 mg/kg/day in drinking water, N=8). On 22nd, 30th, 40th and 50th weeks, 24-hour urine protein was checked. We measured serum adiponectin levels by radioimmunoassay (RIA) and also glomerular optical densities of VEGF expression.
Results: On 40th and 50th weeks, 24-hour urine protein was significantly higher in control OLETF group than pioglitazone and rosiglitazone groups ($P<0.05$). Serum adiponectin levels were higher in pioglitazone and rosiglitazone groups than control OLETF group ($P<0.001$). Optical densities of glomerular VEGF were lower in pioglitazone and rosiglitazone groups than control OLETF group ($P<0.001$).
Conclusion: Serum adiponectin levels were significantly increased and glomerular optical densities of VEGF were decreased after TZD treatment. These results suggested that TZD might be beneficial for the treatment of diabetic nephropathy.

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Impact of physical activity

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Interactive video games as an alternative exercise option for obese adolescents
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Background and Aims: Dance Dance Revolution (DDR; Konami® Corp., Tokyo, Japan), an active video game requiring players to step or jump on a footpad in sync with teen-appropriate music, was incorporated into an 8-week summer weight loss program for obese adolescents to evaluate its effectiveness.
Materials and Methods: Twenty adolescents (15 girls, 5 boys) participated in a 3days/week, 3 hr/day program. Age ranged from 13 to 17 years (mean=15.7, s.d.=0.9), weight from 64.6 to 185 kg (mean=100.9, s.d.=30.1), BMI from 26.3 to 62.8 (mean=36.1, s.d.=8.3), and body fat from 23.7% to 61.9% (mean=43.7, s.d.=9.1). 45% had metabolic syndrome at enrollment. Students were randomized to spend 1 hour/day playing DDR or participating in vigorous group exercise. The remaining 2 hours were spent in group activities that included nutrition lessons, healthy snack preparation, active physical exercise, and motivational training focusing on self-esteem and empowerment. Each participant was loaned a DDR, a PlayStation® 2 (Sony Corp., Tokyo, Japan), and a footpad for home use.
Results: After 8 weeks, there were no differences in weight loss or physiologic parameters between the two groups. Average weight loss was 1.1 kg, ranging from a loss of 10.0 kg to a gain of 1.7 kg. However, a 72.3% attendance rate, with some absences due to family vacations or work schedule conflicts, showed that the program engaged the participants, who reported strong satisfaction with the program and being highly motivated to continue building healthy lifestyles.
Conclusion: Motivation and retention, key challenges to any weight management program, are particularly problematic with obese adolescents. Video games are very popular with teens and therefore active video games that require physical exertion may heighten participation and retention in weight management programs. DDR is a fun and engaging activity that can be played indoors in any weather and offers an opportunity to exercise either in private or with friends. The combined elements of technology, music, movement, and interactive feedback set a platform for success.
Support: Konami Corporation

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Effect of hyperinsulinemia on muscle energy metabolism during exercise in type 1 diabetes
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Background and Aim: Exercise plays an integral part in the management of type 1 diabetes due to its glucose lowering effect. It is usual practice for patients to make adjustments to carbohydrate intake and/or insulin dose to prevent exercise-induced hypoglycaemia. As a result, they frequently exercise under mild or moderate hyperinsulinaemic and hyperglycaemic conditions. However, little is known about the impact of such metabolic conditions on muscle energy metabolism. Hence, we have investigated the metabolic responses to moderate exercise in patients with type 1 diabetes under mild and moderate hyperinsulinaemic conditions that were designed to simulate the metabolic milieu of two real life situations, i.e., exercise in the post-prandial period either with or without major adjustment to pre-meal insulin dose.
Methods: Six male patients with uncomplicated type 1 diabetes [age 37.8 ± 1.9 yr (mean±SD), diabetes duration 12 ± 4 yr, BMI 24.5 ± 2.3, HbA_{1C} 8.0 ± 0.8% and $VO_{2\,max}$ 44.8 ± 3.9 ml/kg/min] were studied for 2 h at rest and during 45 min cycling at 60% $VO_{2\,max}$ in a crossover design on 2 occasions, 2wk apart, after an overnight insulin infusion (i.v) to maintain euglycemia. Hyperinsulinaemic clamp was used to achieve serum insulin concentration of either 25 (LOW trial) or 75 mU/l(MOD trial). Blood glucose was clamped at 8 mmol/l during both experiments. Whole body glucose disposal was determined from glucose infusion rates adjusted for urine glycosuria, whereas whole body substrate oxidation was determined using indirect calorimetry. Muscle biopsies from the vastus lateralis were obtained for the determination of glycogen and lactate concentrations.

Results: Serum insulin concentrations were higher (P< 0.001) in the MOD trial, both at rest (68.9 ± 4.0 vs. 26.5 ± 2.3 mU/l) and during exercise (92.3 ± 4.3 vs. 33.6 ± 1.7 mU/l). Plasma free fatty acids were suppressed in both trials, but more so in the MOD trial (0.10 ± 0.05 vs. 0.24 ± 0.12 mmol/l, P <0.05). During the MOD trial whole body glucose disposal increased by ~4 fold at rest (0.80 ± 0.06 vs. 0.21 ± 0.01 g/min) and by ~2.5 fold during exercise (1.57 ± 0.28 vs. 0.60 ± 0.27 g/min, P < 0.001) when compared to the LOW trial. Carbohydrate oxidation increased by ~15% (2.65 ± 0.27 vs. 2.28 ± 0.27 g/min, P < 0.05) whereas fat oxidation was supressed by ~50% (0.15 ± 0.05 vs. 0.30 ± 0.14 g/min, P=0.08), during exercise in the MOD trial as opposed to the LOW trial. There was no difference in muscle glycogen concentration before (417 ± 54 vs. 410 ± 62 mmol glucosyl units/kg dm) and after exercise (258 ± 59 vs. 242 ± 46 mmol glucosyl units/kg dm), in the LOW and MOD trials respectively. As a result muscle glycogen utilisation was similar in the 2 trials (3.5 ± 1.4 and 3.7 ± 1.7 mmol glucosyl units/kg dm/min, respectively). Muscle lactate concentration increased by 3 to 4 fold after exercise (LOW: 6.1 ± 4.1 to 19.4 ± 10.5, MOD: 6.0 ± 3.4 to 26.8 ± 5.5 mmol/l, NS).
Conclusion: Moderate hyperinsulinaemic hyperglycaemia greatly increases whole body glucose disposal and carbohydrate oxidation without affecting muscle glycogen utilisation. In light of the disproportionate increase in glucose disposal (~2.5 fold) to carbohydrate oxidation (~15%), these data suggest that substantial amounts of exogenous carbohydrate are required to maintain euglycaemia during exercise under moderate hyperinsulinaemic conditions in patients with type 1 diabetes.
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Endothelial function and biochemical vascular markers in first-degree relatives of type 2 diabetic patients; the effect of exercise training
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Background and Aims: Endothelial dysfunction (ED) is associated with the presence of atherosclerosis. However, ED is also considered a sign of the early vascular changes preceding atherosclerosis. We sought to explore whether impaired endothelial function is already present in healthy subjects at increased risk of developing type 2 diabetes. Furthermore we aimed to assess the impact of short term lifestyle intervention (10 weeks endurance exercise) on the potentially primary defects of endothelial function.
Materials and Methods: Twenty-nine healthy but insulin resistant first-degree relatives of patients diagnosed with type 2 diabetes (33 ± 5 years, BMI 26.3 ± 1.6 kg/m^2) were compared with 19 control subjects without a family history of diabetes (31 ± 5 years, BMI 25.8 ± 3.0 kg/m^2).
Results: At baseline the von Willebrand factor was significantly increased in the relatives (P<0.05). Furthermore, mannose binding lectin (MBL) (P=0.06), s-ICAM (P=0.08), and osteoprotegerin (OPG) (P=0.08) tended to be increased in relatives. The following markers of endothelial function were comparable at baseline: flow-mediated vasodilation, C-reactive peptide (CRP), plasminogen activator inhibitor-1 (PAI-1) and s-VCAM. Exercise training resulted in a decrease in MBL (P=0.02) and OPG (P<0.01) in relatives only, whereas other biochemical markers were unaffected in both groups. Moreover, the relatively high intensity exercise training tended weakly to reduce flow-mediated vasodilation in relatives (P=0.15).
Conclusion: Healthy subjects predisposed for type 2 diabetes show only minor signs of endothelial dysfunction. Under these almost normal vascular conditions exercise training had little effect on endothelial function. However, hard physical training administered to previously unfit individuals may affect endothelial function negatively.

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Determinant factors of elasticity improvement in response to brief moderate exercise in a high risk population
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Introduction: The elasticity of blood vessels in the body affects the shape of the blood pressure waveform. HDI's waveform analysis methodology is capable of measuring C1-Large Artery Elasticity (Capacitive Compliance) and C2-Small Artery Elasticity (Reflective Compliance). The acquisition of calibrated radial artery blood pressure waveform data involves the coordinated use of a blood pressure cuff placed on the left upper-arm and a piezoelectric-based, direct contact, acoustical sensor placed over the right radial artery adjacent to the styloid process of the radius (by the wrist). The cuff systolic and diastolic pressures are used to calibrate the data of radial artery waveform into units of pressure based on the median high and low values contained in a 30-second collection of blood pressure waveform data. No studies have yet analyzed the relationship between such evaluation and brief moderate exercise.
Aims: 1. To determine the different types of response to a standardized test of moderate exercise. 2. To determine their correlation with age, anthropometric parameters and hypertensive, diabetic and smoking status.
Methods: N=25 patients, 32-80 yrs (52,76 ± 2,77), 18/7 male/female ratio, 11 hypertensive, 9 with hyperglycemia, and 6 smokers.Metabolic Syndrome (MS) (n=8): ATP III criteria. Metabolic parameters: creatinine, glycemia, triglycerides, LDL, HDL, total cholesterol, assessed with HITACHI autoanalyser. HbA1c: HPLC. Large (C1) and small (C2) artery elasticity index (ml/mmHgx100), systemic vascular resistance (dyne.sec.cm5), total vascular impedance (dyne.sec.cm5). HDI/PulseWave CR-2000, normal values according to age and sex (standard tables by Hypertension Diagnostics Inc. CVProfilor). Six-minute-walk: constant slope (7%) and variable speed: 0,9–6,9 mph (4,84 ± 0,31) Statistical analysis: Kolmogorov-Smirnov, Mann-Whitney's U, Pearson Chi-Square and Student's T tests.
Results: 1 – The prevalence of significant improvement (>10%) of large (C1) artery elasticity was 18,2% (N=4/22) after 2 minutes, and 43,5% (N=10/23) after 5 and 10 minutes.
2 – With respect to small (C2) artery elasticity, we found a significant improvement of 22,7% (N=5/22) after 2 minutes, 39,1% (9/23) after 5 minutes and 45,8% (11/24) after 10 minutes.
3 – The determinant factors predictive of a favourable response in C1 at 10 minutes were: age (59,60 ± 3,97 years in the responsive group vs 48,86 ± 3,55; p=0,036), sex (71,4% of women showing significant improvement vs 29,45% of men; p<0,05), LDL levels (105,10 ± 9,28 vs 138,14 ± 7,64 mg/dl; p=0,009) and non-HDL levels (130,10 ± 9,27 vs 160,14 ± 9,33 mg/dl; p=0,042). Diabetic, hypertensive and smoking status showed no particular influence.
4 – The sole determinant factor predictive of such favourable response in C2 could be the presence of MS (62,5% of those with MS had a favourable response vs 37,5% of those in the control group without MS). The small sample number could explain the fact that this difference did not reach statistical significance.
Conclusions: 1 – The stiffness of large and small arteries is susceptible of improvement in response to brief moderate exercise.
2 – This improvement is progressive after finished exercise, at 2, 5 and 10 minutes.
3 – For large arteries, aged women with low LDL cholesterol levels are the most benefited by this sort of exercise.
4 – For small arteries, patients with Syndrome X could be the most benefited by exercise.

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Physical activity in children is centrally regulated: evidence for an 'Activitystat' (The EarlyBird Study)
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Background and Aims: Diabetes is closely related to obesity. Obesity is attributable to varying degrees of over-feeding and under-activity, and will result from an imbalance as small as 2%. Appetite is under complex central regulation (the 'appestat'), but there is little understanding of what controls physical activity (PA). Here we test the hypothesis that the activity of children is centrally, rather than environmentally, regulated (the 'activitystat').
Materials and Methods: MTI-Actigraph accelerometers were used to record weekly PA in two groups of healthy children: Group 1 comprised 215 children aged 9.0 SD 0.9y from three schools (S) differing widely in the pro-

vision of timetabled physical education (PE). S1 provided 9.0 hours per week, S2 and S3 only 2.2 and 1.8 hours respectively. Group 2: 300 of the EarlyBird cohort examined at 4.9 SD 0.3y and again at 5.9 SD 0.3y.
Results: Girls were systematically less active than boys in both groups. Correlations in Group 2 for schoolday/weekend day activity (B r= 0.51 and G r= 0.52, p<0.001) and year-on-year activity (B r= 0.55 and G r= 0.49, p<0.001) were high for both genders. Mean PA levels in Group 1 during school hours were predictably highest in S1, but what PA S2 and S3 lacked in school, they made up for - with striking precision - out of school. Thus, total daily activity was the same irrespective of the school attended (B: S1=34.7, S2=39.1, S3=33.8 $x10^5$ units/wk, G: S1=30.5, S2=32.4, S3=34.0 $x10^5$ units/wk), and <1% of the variance in total PA could be explained by the five-fold difference in timetabled PA (p>0.5). We then compared walking to school v transport by car in Group 2. The PA cost of car transport represented only 2% of the total PA recorded in a week. Even then, over 90% of it was 'recovered' in more PA after-school among those driven by car, so that the total weekly PA recorded by the two groups was identical (walk 37.56, car 37.60 PA units/wk, p= 0.97). Finally, the mean weekly PA recorded by EarlyBird children at 5.9y in Plymouth was identical to that recorded by an independent study of 5.8y-olds using the same accelerometers in Glasgow, 800 km away (37.4 v 37.4 $x10^5$ units).
Conclusion: The data are consistent with the central regulation of PA in young children. Despite widely differing opportunity, variation in PA appears to lie with the child, and not his/her environment. While there is wide variation in PA *between* children, there is remarkable consistency *within* them, irrespective of routine, location, or culture. The child who is inactive at school is inactive at the weekend and remains so year on year, and the child who lacks activity during school hours makes up for it afterwards. The correlations within groups over time, and the similarities between groups irrespective of environment, point to the operation of an 'activitystat' for energy expenditure that corresponds to the 'appestat' for energy intake. Efforts to increase provision for physical activity, in the belief that children will expend more energy, may be overridden by a central control system that adjusts activity to a biological rather than environmental setting. These findings may be important to governments and health strategists concerned with the rising tide of obesity and diabetes in young people. They also imply novel neuro-humoral pathways - regulation involves signalling.
Support: Diabetes UK, Diabetes Foundation, Child Growth Foundation, Abbott, Ipsen, GSK, Astra-Zeneca, Unilever Research

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Physical activity of young UK children does not impact on their metabolic health – an objective assessment. (The EarlyBird Diabetes Study)
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Background and Aims: Childhood obesity is a major threat to diabetes risk, and physical under-activity is perceived to be an important cause. However, perceptions of the physical activity (PA) of children are mostly derived from data based on self-report or observation, both of which are imprecise and unreliable. This study sought to characterize, objectively, the habitual daily PA of 7y olds and relate it to gender, UK recommendations, adiposity and metabolic health.
Subjects and Methods: EarlyBird is a non-intervention, prospective, single cohort study that is closely monitoring the lifestyle, physical development and metabolic health of 300 healthy children from school entry at 5y (2000–2001) to young adulthood at 16y. This report is based on the 209 children (124 boys, 85 girls) who had a complete data set at their 3rd annual visit (age 6.9 +/– 0.3y). Main outcome measures include: Physical activity - Electronic MTI accelerometers were worn for seven consecutive days (five school days and the weekend), sampling movement 600 times per minute and recording approximately 5000 individual minutes of data for each child. Calibration studies defined three intensities of PA: low (equivalent to walking <1 km/h), medium (walking 1–4 km/h) and high (>4 km/h). Adiposity – BMI and total body fat% by dual X-ray absorptiometry (DEXA). Metabolic health – Fasting blood samples for insulin resistance by HOMA-IR, cholesterol/HDL ratio and triglycerides.
Results: The girls had higher BMI (17.1 v 16.0 kg/m^2, p<0.001), %body fat (22.3 v 14.6%, p<0.001), insulin resistance (0.42 v 0.29 HOMA-IR units, p<0.001), cholesterol/HDL ratio (2.78 v 2.61, p=0.01) and triglycerides (0.60 v 0.52 mmol/L, p=0.005) than boys. The girls also recorded less total PA (5.04 v 5.63 counts × 10^5/day, p<0.001) and less high-intensity PA (1.72 v 2.20 counts × 10^5/day, p<0.001). Forty three percent of boys and 17% of girls met UK recommendations (1 hour/day or more of at least 'high' intensity PA) Boys, but not girls, who met the recommendations had lower %body fat than those who did not meet the recommendations (12.8 v 15.9%, p=0.01). However, there were no differences in BMI (p>0.17), insulin resistance (p>0.42), cholesterol/HDL ratio (p>0.63) or triglycerides (p>0.09) between the two groups in either sex. In the boys only, there were modest, but significant, inverse correlations between total PA and BMI (r = –0.19, p=0.03) and between total PA and % body fat (r = –0.27, p=0.002). There was no correlation between total PA and any metabolic risk factor in either sex (r = –0.09–0.01, all p>0.40).
Conclusion: At 7y, girls systematically undertake less physical activity than boys. Physical activity at this age does not appear to relate to metabolic health. Adiposity in boys is the only measure that distinguishes children achieving UK Government recommendations for physical activity, and the only measure that correlates with activity. The fact that most children do not achieve the prescribed amount does not necessarily imply under-activity. The recommendations as to the amount of activity children should undertake are arbitrary. Further studies as these children grow older will determine whether physical activity impacts at all on metabolic health.
Support: Diabetes UK, Child Growth Foundation, GSK, Abbott Laboratories, Astra-Zeneca, Ipsen

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Monitoring kidney function in type 2 diabetic patients with incipient and overt diabetic nephropathy

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Background and Aims: To evaluate the agreement between GFR and rate of decline in GFR estimated from the MDRD equation (based on s-Cr, age, gender and race) or estimated from the Cockroft-Gault formula (CG) and measured by the plasma clearance of ^{51}Cr-EDTA
Materials and Methods: A cohort of type 2 diabetic patients with microalbuminuria n=156, 117 males, mean (SD) age 55 (7), BMI 29.8 (4.4) kg/m^2 followed for 8 years with 4 measurements of GFR, and another cohort of type 2 diabetic patients with overt diabetic nephropathy n=227, 167 males, mean (SD) age 57 (8), BMI 30.0 (5.3) followed for 6.5 (range 3–17) years with 7 (3–22) measurements of GFR
Results: For patients with microalbuminuria mean (SD) baseline GFR (ml/min/1.73 m^2) was 117 (24) (range 31–178) measured, and 92 (20) estimated (MDRD) or 103 (24) estimated CG ($p<0.001$). The difference between an individually estimated value and measured GFR (the 95% limits of agreement) were –66 to 20 ml/min/1.73 (MDRD) and –59 to 31 (CG). Rate of decline in GFR (mean (SD)) was 4.1 (4.2) ml/min/year measured, and 2.9 (2.8) MDRD or 3.4 (3.2) CG ($p<0.001$). For patients with overt nephropathy baseline GFR was 84 (30) (range 20–175) measured and 73 (24) MDRD or 81 (28) CG with 95% limits of agreement of –47 to 25 (MDRD) and -39 to 33 (CG) ($p<0.05$). Rate of decline in GFR was 5.2 (4.1) measured, and 4.2 (3.8) MDRD, and 4.6 (4.1) CG ($p<0.001$).
Conclusion: glomerular filtration rate is significantly underestimated with wide limits of agreement by the MDRD equation as well as by the Cockroft-Gault formula. Particularly in microalbuminuric (hyperfiltering) patients. The rate of decline in GFR is also significantly underestimated with both equations. This makes the GFR estimations unacceptable for monitoring kidney function in individual or groups of type 2 diabetic patients.

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Contrasting clinical and cardiovascular risk status between early and later onset type 2 diabetes

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Background and Aims: The prevalence of type 2 diabetes (T2DM) is increasing rapidly in Ireland, and the age of presentation is falling. This is closely linked to the current obesity epidemic. Differences in the clinical characteristics of younger Irish patients with T2DM compared to older patients have not previously been examined. Our objective was to compare the characteristics of younger patients with T2DM (diagnosed at age <40 years) with older patients (diagnosed at age 50–70 years).
Materials and Methods: We identified 149 younger patients with T2DM (aged 35.16 +/– 0.39 years), from our diabetes clinic database, and compared them with 217 older T2DM patients (aged 61.61 +/–0.38 years) randomly identified from the same database. Clinical and labaratory data are prsented in the table below. Figures in brackets represent SEM
Results:

	Younger T2DM (n=149)	Older T2DM (n=217)	P Value
Age (years)	35.16 (0.39)	61.61 (0.38)	
BMI (kg/m^2)	32.28 (1.65)	30.66 (0.5)	0.03
HbA1C at Diagnosis (%)	9.38 (0.21)	8.75 (0.14)	< 0.01
Current HbA1C (%)	8.02 (0.19)	7.35 (0.09)	< 0.01
Total Cholesterol (mmol/l)	4.98 (0.12)	4.83 (0.07)	NS
LDL (mmol/l)	2.83 (0.07)	2.79 (0.06)	NS
HDL (mmol/l)	1.07 (0.03)	1.17 (0.02)	< 0.01
Total Cholesterol/HDL ratio	4.83 (0.11)	4.23 (0.06)	< 0.01
Triglyceride (mmol/l)	2.60 (0.22)	2.09 (0.13)	0.04
Systolic BP (mm Hg)	124 (1.23)	136 (1.5)	NS
Diastolic BP (mm Hg)	80 (0.91)	78 (0.81)	NS

Conclusion: Younger patients with T2DM are more obese, hypertriglyceridaemic, with lower HDL and higher Total Cholesterol/HDL Cholesterol ratio. They also present with worse initial hyperglycaemia and continue to have poorer ongoing glycaemic control than older patients from the same clinic, in spite of exactly similar treatment and follow up protocols. This phenomenon is a new and serious challenge for diabetes management. Younger patients with T2DM present a higher cardiovascular risk profile than older patients, and will also face a longer disease duration and possibly severity of hyperglycaemia. Treatment of younger patients with T2DM is likely to require early aggressive lifestyle modification and intensive multi-factorial therapy in order to prevent long-term complications.

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Oesophageal dysmotility, abnormal gastric emptying and autonomic neuropathy have different effects on glucose haemostasis

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Background and Aims: Amongst diabetic patients, glucose haemostasis is believed to be affected by abnormal gastric emptying, autonomic neuropathy, and perhaps by abnormal oesophageal function. To evaluate this issue, we continuously recorded subcutaneous glucose concentrations during 52 hours and related the findings to oesophageal motility, gastric emptying and autonomic neuropathy in 20 diabetic patients. The aim was establish whether glucose haemostasis is affected differently by disturbances in these 3 different systems.
Materials and Methods: Diabetic patients were investigated by oesophageal manometry (n=14; dysmotility and peristaltic speed), gastric emptying scintigraphy (n=20;Technetium-99; whole meal; abnormal: T50 > 2 SD in normal controls) and heart rate variation during deep breathing (test of parasympathetic function; n=18; E/I ratio; abnormal: age corrected value <-1.64 SD). Continuous subcutaneous glucose concentrations were measured during 52 hours using Minimed Continuous Glucose Monitoring system (CMGS). On the second day, a carbohydrate rich breakfast was provided.
Results: 8 of 14 patients had oesophageal dysmotility, 11/20 had abnormal gastric emptying and 9/18 abnormal E/I ratio. After breakfast during the first hour, glucose levels rose similarly in patients with and without abnormal gastric emptying. However, thereafter, glucose levels decreased in those with and continued to rise in those without abnormal gastric emptying. Accordingly, median glucose level was clearly lower in patients with than in those without abnormal gastric emptying 2.5 hours after breakfast (9.1 [4.2–12.5] mmol/l vs. 14.3 [11.2–17.7] mmol/l; P <0.05). In contrast, patients with abnormal oesophageal manometry showed slowly increments in glucose (abn vs. norm. manometry; 10 mmol/l vs. 14 mmol/1 1 hour after breakfast) that continuously increased up to 4 hours after breakfast whereas glucose decreased 2 hours after breakfast in those with normal manometry. In agreement, the peak for glucose was delayed in patients with abnormal oesophageal manometry vs. those with abnormal gastric emptying (3.5 [1,8–4.9] hrs vs. 1.8 [1,1–2) hrs; P=0.08). There was a significant correlation between mean peristaltic speed of oesophagus and T50 (r = –0.67; P = 0.02); low speed indicated delayed gastric emptying. There was no correlation between E/I ratio and glucose values after breakfast. However, the glucose variation during the 52 hours of registration was significantly higher in those with an abnormal E/I ratio versus those without (Coefficient of variation day-night glucose values: 41 [46–49] vs. 28 [27–34] %; P=0.008).
Conclusions/ Interpretation: Abnormal gastric emptying is associated with diminished glucose uptake from the gut after a meal, abnormal oesophageal manometry is associated with a low and delayed uptake of glucose after a meal, whereas parasympathetic neuropathy is associated with unstable glucose values. Our study argues for caution regarding dosing of insulin in patients with abnormal gastric emptying, insulin injection may be delayed in patients with abnormal oesophageal manometry and autonomic neuropathy seems to be an important factor behind brittle diabetes.
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Different influences of type 2 diabetes on bone mass of postmenopausal women

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Background and Aims: _he influence of diabetes mellitus type 2 on bone metabolism is a controversial subject, however most of the studies suggest an association to increased bone mineral density (BMD).The purpose of the present study was to investigate the effect of diabetes on bone mineral density (BMD) of postmenopausal women belonging to different age segments.
Materials and Methods: The BMD of femoral neck (FN) and trochanter (TRO) was measured by dual-energy X-ray absorptiometry in 89 postmenopausal diabetic women aged 50–60 (D-MID) who were compared to 267 healthy controls (H-MID) and in 39 diabetic women aged 70–80 (D-OLD) compared to 117 healthy controls (H-OLD). The control groups were matched for age, number of years since menopause and BMI. The age of D-MID was 55.9 ± 2.9 years (mean± 1SD), the age at menopause 50 ± 2.6, years since menopause 5.9 ± 3.4, BMI 26.8 ± 4.2 kg/m^2, diabetes duration 6.8 ± 3.6 years, HbA1c levels 7.6 ± 0.9% whereas the respective values of group D-OLD were 74.3 ± 3.3, 50 ± 3, 24 ± 3.9, 28.9 ± 3.6, 19.6 ± 3.1 and 7.5 ± 0.6. The duration of diabetes in all patients exceeded 75% of the postmenopausal time period. None of the women had ever received any medication or had suffered from any disease affecting bone metabolism.
Results: Absolute BMD values as well as the age-matched ones (Z scores) of D-MID group were significantly higher compared to H-MID group in both anatomic sites measured. [FN: (0.81 ± 0.1 vs 0.76 ± 0.1 gr/cm^2, p=0.002, Student's t-test) and (0.05 ± 1.2 vs –0.32 ± 1 SDs, p<0.001), TRO: (0.66 ± 0.12 vs 0.62 ± 0.11 gr/cm^2, p=0.001) and (0.19 ± 1.27 vs -0.7 ± 1.38 SDs, p<0.001)]. No significant differences were observed neither in absolute nor in age-adjusted BMD values between D-OLD and H-OLD groups [FN: (0.68 ± 0.12 vs 0.67 ± 0.12 gr/cm^2, NS), and (0.67 ± 1.08 vs 0.58 ± 1.11 SDs, NS), TRO: (0.56 ± 0.97 vs 0.57 ± 0.1 gr/cm^2, NS) and (0.13 ± 0.98 vs 0.2 ± 1.07 SDs, NS)]. The proportions of osteopenia and osteoporosis were significantly lower in D-MID than in H-MID women (47 and 16% vs 58 and 20%, p<0.01, chi-square test). No significant differences were observed regarding such proportions between the D-OLD and H-OLD groups. In D-OLD, H-OLD and H-MID women both FN and TRO BMD values were positively correlated to BMI [(D-OLD: r=0.41 and 0.42 p<0.05), (H-OLD: r=0.28 and 0.46 p<0.01), and (H-MID: r=0.23 and 0.75 p<0.01 respectively, Pearson's coefficient). In D-MID women only TRO BMD values were positively correlated to BMI (r=0.29, p<0.01). No correlation were observed between the BMD values of any diabetic group and either the duration of diabetes or HbA1c levels.
Conclusion: Middle-aged postmenopausal women with type 2 diabetes present higher bone mineral density values in both trabecular and mixed bone compared to age-matched healthy controls. However, this favourable effect is not observed in those women aged over 70 with longer diabetes duration. It remains to be settled whether these findings reflect an effect of either advanced age or longer diabetes duration. The degree of glucose control does not seem to influence bone mineral density.

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Cystic fibrosis related diabetes in a cohort of 243 adults

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Background and Aims: This retrospective cohort study was done to evaluate the prevalence of cystic fibrosis related diabetes (CFRD) and to identify predictive factors of its development.
Materials and Methods: We included 243 adult patients with CF who attended the CF centre, Cochin hospital, Paris, France, from December 1997 to April 2004. Their median age was 27 (18–60 years). 124 (51%) were female and 198 (81.5%) had exocrine pancreatic insufficiency (PI). Excepted in patients already diabetic, glucose tolerance was evaluated by the oral glucose tolerance test (OGTT). Diagnostic criteria of impaired glucose tolerance (IGT) and diabetes were those of WHO. Predictive factors in PI patients were investigated using survival analysis (Cox's proportional hazards models).
Results: 88 patients (36.2%) had CFRD, all of them with PI and 37 patients (15.2%) had IGT, 2 of them being pancreatic sufficient. Median age at CFRD diagnosis was 21.5 (6.7–49 years) and 54.5% were female. Only 33% of CFRD patients were diagnosed on the fasting glycaemia, the majority being discovered on the OGTT. At the last visit, 60% were treated with insulin. No CFRD patient had a neuropathy. Concomitant retinopathy and nephropathy were diagnosed in 2 patients, 8 and 13 years after CFRD discovery. In 2 other patients, isolated nephropathy appeared 5 and 10 years after CFRD diagnosis. Pulmonary insufficiency was more severe in diabetics compared with normoglycemic patients, as attest the mean forced expiratory volume in one second (p<0.001).
Predictive factors in the 198 PI patients were a liver cirrhosis (RR = 2.00 [1.05–3.80], p=0.04) and a meconium ileus history (RR = 1.90 [1.05–3.45], p=0.03). A CF diagnosis before 18 years old (RR = 2.50 [0.99–6.30], p=0.051) was marginally associated with CFRD.
Neither the female sex (RR = 1.40 [0.90–2.20], p=0.11), nor a severe versus a moderate pulmonary insufficiency (RR= 1.70 [0.90–3.50], p=0.11), nor the genotype (p=1.00) were significantly associated with CFRD.
Conclusion: CFRD developed only in PI patients and the risk of CFRD in these PI patients was increased in patients with cirrhosis, meconium ileus or in CF diagnosed in childhood. As CFRD was related to the severity of respiratory insufficiency and is at risk of microvascular complications, we advocate an OGTT once a year in CF adult patients.

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Preservation of endogenous insulin production in new-onset type 1 diabetes by treatment with DiaPep277, an immunomodulatory peptide: a randomised, double-blind, phase II trial

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Background and Aims: It has previously been shown that an immunomodulatory peptide, DiaPep277, derived from the human hsp60, arrested beta cell destruction in diabetic prone NOD mice. This phase II study was designed to evaluate first the effect of DiaPep277 on preservation of functional beta-cells, and further to look at the long-term effects of ongoing or cessation of treatment. The study was a single-center, randomised, double-blind, placebo-controlled phase II study in patients who were at least 16 years old, with newly diagnosed (<6 months) type 1 diabetes.
Materials and Methods: 35 male patients, with fasting basal C-peptide concentrations above 0.1 nmol/L were assigned to subcutaneous injections of either 1 mg DiaPep277 (N=18) or placebo (N=17). Treatment was administered at entry, 1, 6, and 12 months later, as a subcutaneous injection. After the last injection, the patients were followed for additional 6 months without treatment, to a total period of 18 months. The primary endpoint was preservation of glucagon-stimulated endogenous insulin secretion, tested by C-peptide serum concentration in a 20-minutes test. Secondary endpoints were metabolic control as tested by HbA1c and daily insulin dose requirement. Twenty-seven patients completed the full treatment and follow-up period of 18 months, and were offered an extension of the study protocol, without unblinding the patients' randomization codes. The placebo arm patients were crossed over to treatment with 1 mg DiaPep277 while those on DiaPep277 were randomized to either placebo or 1 mg DiaPep277. During the extension period, patients were treated at 0, 3, 6 and 9 months and followed up to month 12. The patients were thus treated and followed up for a total period of 2.5 years, with an average break period of 6 months between the 2 stages of the study.
Results: At staging, patients were an average of 96 days from diagnosis with type 1 diabetes, their average age was 25 years and average BMI was 22. Their fasting basal C-peptide was 0.48 nmol/L, and their HbA1c was 6.8%. After 18 months, mean stimulated C-peptide concentrations had significantly dropped in the placebo group from a baseline of AUC=15.6 nmol*min/L to AUC=9.2 nmol*min/L, while in the DiaPep277 group the drop was minimal, from 11.7 nmol*min/L to 10.6 nmol*min/L (P=0.028). Mean HbA1c was similar, 7.2% vs. 7.3% at 18 months. However, 50% of DiaPep277-treated patients maintained HbA1c below 7% compared to only 30% of placebo-treated, while exogenous insulin dose was higher in the placebo than in the DiaPep277 group. At study extension, patients who were continuously treated with DiaPep277, had better but not statistically significant preservation of beta cell function: AUC dropped (from the study's original baseline) by 3.6 nmol*min/L, compared to those patients switched to placebo, whose AUC dropped by 6.7 nmol*min/L.
Conclusion: Treatment of newly diagnosed type 1 diabetes with DiaPep277 preserves endogenous insulin production, at significant levels. It also results in a better chance to achieve target metabolic control, without increasing insulin dosage. Treatment was well tolerated and no safety or concerns were observed after repeated exposure to the peptide. However, to maintain the therapeutic effect, treatment has to be continued.

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Endothelium and diabetes

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***De novo* emergence of insulin responsiveness in human aortic endothelial cells and T-lymphocytes in response to hyperglycemic or saturated free fatty acid exposure**

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Background and Aims: Human aortic endothelial cells (E-cells) and T-lymphocytes (T-cells) are important components in pathogenesis of atherosclerotic plaque. These two cell types have certain similarities in that neither of the cells is insulin responsive but upon incubation in 30 mM glucose become activated and produce reactive oxygen species (ROS) and exhibit lipid peroxidation. Furthermore, although activated T-cells develop insulin receptors and insulin degrading enzyme (IDE) and metabolize glucose in response to insulin, it is not known if E-cells behave similarly.
Materials and Methods: We therefore studied activation of these cells in response to various doses of glucose (5, 15, 30 mM) and saturated (palmitate) and unsaturated (oleic, linolenic, and linoleic) free fatty acids (FFA) at 1, 50, and 500 μM at 37°C for up to 72 hours. We measured emergence of insulin receptors by flow cytometry (in T-cells) or ^{125}I-insulin binding and ^{14}C-deoxyglucose uptake in response to insulin and assessed the expression of insulin receptor, GLUT1 and GLUT4 by Western blotting (in E-cells).
Results: Both E-cells and T-cells in response to 30 mM glucose, but not 5 mM glucose or 25 mM mannitol exhibited time- and temperature-dependent emergence of insulin receptors. 2-^{14}C-deoxy-D-glucose uptake in E-cells was also noted in response to insulin only at 15 and 30 mM glucose but not at 5 mM glucose or 25 mM mannitol. Insulin receptor expression in E-cells was increased after incubation of the cells with 30 mM glucose after 24 and 48 hours of incubation by 30% ($P<0.05$) and 45% ($P<0.05$), respectively, as compared with 5 mM glucose values. Immunoblotting experiments also showed that incubation of E-cells cells in 30 mM glucose was accompanied by a significant elevation in expression of GLUT1 (by 160% and 250% after 24 and 48 hours, respectively) and GLUT4 (by 170% and 173% after 24 and 48 hours, respectively) as compared with 5 mM glucose values. Results of incubation of E-cells and T-cells with palmitate at 1, 50, 500 μM and linolenic acid (500 μM) on emergence of GLUT4, insulin receptor, IRS1, ROS and lipid peroxidation (by TBA assay) are shown below. Similar to linolenic acid, negative results were also obtained with other unsaturated FFA.

		T-CELLS *Palmitic Acid*		T-CELLS *Linolenic Acid*		E-CELLS *Palmitic Acid*		E-CELLS *Linolenic Acid*
	1μM	50μM	500μM	500μM	1μM	50μM	500μM	500μM
GLUT 4	0	6 ± 1	13 ± 2	0	0	6 ± 1	13 ± 3	0
Insulin Receptor	0	8 ± 2	22 ± 3	0	0	6 ± 2	17 ± 3	0
IRS-1	0	5 ± 2	11 ± 2	0	0	7 ± 2	11 ± 2	0
ROS	0	10 ± 2	32 ± 4	0	0	14 ± 2	34 ± 4	0
Lipid Peroxidation	0	7 ± 2	24 ± 4	0	0	12 ± 2	29 ± 4	0

Conclusions: We conclude that both E-cells and T-cells, which are normally devoid of insulin receptors once activated with high glucose and saturated FFA become insulin responsive cells and metabolize glucose in a dose-dependent fashion concomitant with elaboration of free radicals. We hypothesize that high glucose and saturated FFA upregulate glucose transporter systems concomitant with emergence of insulin receptors as a defense mechanism to protect these cells from glucotoxicity and lipotoxicity.
Support: GCRC/NIH

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Response of human aortic endothelial cells to diabetic milieu: identification of phenotypic changes and signal transduction mediators

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Background and Aims: Evidence from investigations on diabetic animal models indicate that endothelial cell dysfunction is associated with hyperpermeability, enhanced biosynthetic activities, proliferation and reduced NO bioavailability, that all contribute to diabetes-associated vascular complications. This study aimed to dissect the effects of circulating deleterious factors in type 2 diabetes (elevated glucose concentration, insulin, sera of obese patients) on human aortic endothelial cells (HAEC) in culture by investigating changes in phenotype and expression of signaling molecules such as Ob-R (the long form of leptin receptor), STAT-3 (signal transducer and activator of transcription-3) and extracellular signal-regulated kinase (ERK1/2).
Materials and Methods: HAEC were cultured till confluence in DMEM (with 10% fetal calf serum) supplemented with 30 mM glucose (controls: 5 mM glucose) and in DMEM containing 10% serum of obese type 2 diabetic patients (BMI: 29– 32 $kg \cdot m^{-2}$, circulating glucose: 152–185 mg/dl) (control: 10% serum of normal subjects, BMI: ~19 $kg \cdot m^{-2}$, glycemia: ~80 mg/dl). The structure of HAEC was investigated by electron microscopy, the localization of focal adhesion protein α-actinin was studied by immunofluorescence, and the expression of signaling molecules was examined by immunoblotting using antibodies against the tyrosine-phosphorylated and total proteins.
Results: Compared to control condition, 30 mM glucose induced: (i) a secretory phenotype of HAEC enriched in biosynthetic organelles such as rER and Golgi cisterne, similar to that encountered at diabetic animal models; (ii) augmented expression and reorganization of focal adhesion protein α-actinin, suggesting remodeling of actin filaments crosskinking to the plasma membrane; (iii) ~2 fold increase in phosphorylation of STAT-3 and ERK1/2, contributing to high-glucose intense proliferation and differentiation. Insulin (10–100 nM) stimulation of HAEC cultured in normal serum produced a dose dependent increase in phosphorylation of Ob-R, STAT-3 and 44 kDa ERK, while phosphorylated 42 kDa ERK and total Ob-R were not modified vs. the no insulin condition. The exposure to obese serum produced ~1.3 and ~1.6 fold enhanced phosphorylation of STAT-3 and ERK 1/2, respectively and no modification in both total and phosphorylated Ob-R, compared to HAEC grown in sera of normal subjects.
Conclusion: Taken together, these data indicate that chronic exposure to the components of the diabetic milieu such as glucose, insulin or serum of obese type 2 diabetic patients are associated with abnormal signaling in HAEC that may contribute to the structural and functional abnormalities.

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Decreased brachial artery reactivity and increased CD40L levels in offspring of type 2 diabetics patients

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Background and Aims: Offsprings of type 2 diabetes (T2D) patients have increased predisposition to diabetes, a major risk factor for atherosclerotic cardiovascular disease. Since endothelial dysfunction plays a central role in the development of atherosclerosis, this study was aimed to determine whether differences exist in the endothelial function between young first-degree relatives (FDRs) of patients with T2D and subjects without family history of diabetes (NS).
Materials and Methods: To this purpose, endothelium-dependent (reactive hyperemia; FMD) and -independent (0.5 mg sublingual nitroglycerin; NMD) vasodilation were assessed in the conductance vessels of 56 FDRs and 29 NS (mean age: 30 ± 4.7 and 31 ± 7.2 years, respectively $p>0.05$), using the brachial artery reactivity technique. All subjects in both group were normoglycemic, normotensive, non-smoker and normocholesterolemic.
Results: Baseline diameter (3.3 ± 0.6 vs. 3.1 ± 0.5 mm) and mean reactive hyperemia after cuff deflation were similar in FDRs and in NS ($p>0.05$ for both). In contrast, FMD was significantly lower in FDRs compared to NS (11.1 ± 6.2 vs. 15 ± 7.8%, $p=0.03$). Similarly, NMD was significantly lower in FDRs than in NS (15.7 ± 6.5 vs. 19.7 ± 6.8%, $p=0.001$). Significant differences between the two groups (all<0.05) were present in body mass index

(BMI), as well as in plasma levels of HDL-cholesterol, fibrinogen and haemoglobin A1c and the serum inflammation marker CD40L; however, both FMD and NMD remained significantly lower in FDRs than in NS even after adjustment for these covariate (both<0.05). On multiple linear regression analysis, FMD was significantly predicted by HDL and CD40L (p=0.03 and p=0,02), while none of the these variables was significantly associated with NMD.
Conclusion: FDRs have reduced vascular responsiveness to both endothelium-dependent and -independent vasodilator stimuli. Moreover, FDR status is accompanied by increased proinflammatory "milieu" and a slightly dyslipidemic condition. These findings suggest that abnormalities in vascular reactivity are present at an early stage in individuals at risk of developing type 2 diabetes, even when fasting plasma glucose levels are still within the normal range.

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Thiazolidinediones inhibit apoptosis and heat shock protein 60 expression in human vascular endothelial cells

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Background and aims: This study evaluated direct effects of peroxisome proliferator-activated receptor γ (PPARγ) agonists, including thiazolidinediones (TZDs), on vascular cell apoptosis and related protein expression. We tested the hypothesis that these effects are dependent on ***i)*** the respective agent's structure and ***ii)*** endothelial cells' vascular origin.
Materials and methods: Human endothelial cells, isolated from umbilical veins (*HUVECs*), adult veins (*HAVECs*) or aortae (*HAECs*) were incubated (48 h) with different ligands of PPARγ, including TZDs and the natural PPARγ agonist 15-deoxy-Δ^{12-14}-prostaglandin J_2 (PGJ_2). Subsequently, apoptosis, activation of caspases, and protein expression were determined by DNA fragmentation assays or alterations of mitochondrial membrane potential, FACS-, and Western blot analyses, respectively.
Results: Exposure (48 h) of human umbilical vein endothelial cells (*HUVECs*, n=6) to up to 10 µmol/l troglitazone (TRO), rosiglitazone, pioglitazone, and to up to 50 µmol/l RWJ241947=MCC-555 (RWJ) inhibited (p<0.05) apoptosis by 8–25%, whereas PGJ_2 triggered (50 µmol/l: +400%, p<0.05) endothelial cell death versus control (=100%). Moreover, RWJ (50 µmol/l) completely abrogated TNF-α (2000U/ml) and stearic acid (200 µmol/l) induced apoptosis in *HUVECs*. Similar results were obtained in human adult (saphenous) vein- and aortic endothelial cells, the latter showing no anti-apoptotic response to TRO. In *HUVECs*, TZDs' anti-apoptotic effects inversely correlated (r=-0.95, p<0.01) with increased (p<0.05) expression of the apoptosis-inhibitor bcl-2, whereas PGJ_2-induced apoptosis was associated with upregulation of c-myc (+447%) and E2F-1 (+339%). Additionally, TZDs (by 25–39%) and PGJ_2 (–70%) reduced (p<0.05) expression of heat shock protein 60 (hsp60) showing no correlation with apoptosis (r=0.14, n.s.).
Conclusions: Modulation of apoptosis by PPARγ agonists differs in endothelial cells dependent on their vascular origin and the agonists' structure. Thiazolidinediones' ability to reduce both, endothelial apoptosis and hsp60 expression could well add to beneficial vascular effects attributed to these oral antidiabetic drugs.

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Short-term intensive insulin therapy improves insulin resistance and endothelial dysfunction in Hispanic type 2 diabetic individuals

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Background and Aims: The effects of intensive insulin therapy on insulin resistance and on endothelial dysfunction and vascular inflammation were examined in 16 T2DM (age=47 ± 5 y; 11F/5M; BMI=32 ± 2 kg/m^2) randomized to either continuous subcutaneous insulin (aspart) infusion (CSII, Minimed/Animas pump, n=10) or to multiple daily injections (MDII, glargine/aspart) (IT), and in 10 (5M/5F) age, wt-matched T2DM on conventional therapy (NPH/aspart twice daily = CT).
Material and Methods: Each subject received a 180-min euglycemic insulin (80 mU/m^2.min) clamp to quantitate insulin-mediated glucose disposal (M). Plethysmography was used to assess changes in forearm blood flow (FBF) after (i) 5 min of reactive hyperemia (RH); (ii) brachial arterial infusion of acetylcholine (Ach: 7.5,15&30 µg/min) and (iii) of sodium nitroprusside (SNP: 3&10 µg/min). HbA_{1c}, fasting plasma glucose (FPG), lipids, adiponectin (ADP) and endothelial markers were measured at baseline and after 9 months of therapy.
Results: In IT, HbA_{1c} (9.0 ± 0.4 vs. 7.0 ± 0.1%), FPG (196 ± 19 vs. 126 ± 9 mg/dl), TG (160 ± 25 vs. 112 ± 24 mg/dl) & VLDL (130 ± 29 vs. 51 ± 8 mg/dl) decreased (p<0.01); in CT, HbA_{1c} (8.5 ± 0.4 vs. 8.9 ± 0.6), FPG (187 ± 20 vs. 177 ± 21), TG (162 ± 27 vs. 143 ± 20) & VLDL (128 ± 26 vs. 114 ± 21) did not change after therapy. Plasma ADP increased from 6.4 ± 2.4 to 13.5 ± 4.2 ng/ml and hsCRP (6.5 ± 1.4 vs. 1.3 ± 0.4 mg/L), TNF-α (2.2 ± 0.4 vs. 0.5 ± 0.4 mg/L), IL-6 (3.7 ± 0.4 vs. 0.8 ± 0.2 pg/L), VCAM (501 ± 24 vs. 388 ± 66 ng/ml) & endothelin (1.1 ± 0.4 vs. 0.5 ± 0.2 pg/ml) all decreased (p<0.01) in IT; in CT there were no significant changes in ADP, 6.5 ± 3.2 vs. 7.2 ± 2.4 ng/ml; hsCRP, 6.1 ± 1.1 vs. 5.3 ± 0.6; TNF-α, 1.3 ± 0.3 vs. 1.9 ± 0.5; IL-6, 2.8 ± 0.4 vs. 2.3 ± 0.6; VCAM, 530 ± 35 vs. 515 ± 55 and endothelin 1.2 ± 0.4 vs. 0.9 ± 0.3
M increased in IT (4.0 ± 1.1 vs. 6.0 ± 1.3, p<0.05), but not in CT (5.8 ± 1.1 vs. 5.0 ± 0.7 mg/kg · min). FBF (ml/100 ml · min) and % rise in FBF during dynamic testing are shown below:

IT	FBF	RH*	Ach 7.5*	Ach 15*	Ach 30*	SNP 3*	SNP 10*
BASELINE	2.5±.1	171±27	126±23	200±30	321±60	168±18	247±29
9 MONTHS	2.3±.1	270±12	235±21	290±7	360±14	190±17	305±33
CT							
BASELINE	2.4±.1	208±28	138±22	181±19	222±20	151±16	250±20
9 MONTHS	2.5±.1	160±20	110±10	160±10	190±10	150±10	200±10

* = p <0.01 baseline vs. 9-mo. & between groups

Conclusions: Short-term intensive insulin therapy rapidly corrects the metabolic imbalance and is accompanied by improved endothelial dysfunction and reduced inflammation in Hispanics with type 2 diabetes. These may represent some of the underlying mechanisms responsible for the beneficial cardiovascular effects of intensive insulin therapy.
Support: American Diabetes Association and Takeda Pharmaceuticals of North America

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The protective effect of rosuvastatin in human umbilical endothelial cells exposed to constant or intermittent high glucose-induced oxidative stress, apoptosis and adhesion molecules expression

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Background and aims: The exposure of vascular endothelium to high glucose levels results in increased oxidative insult and to vascular dysfunction in diabetes. We have investigated the effects of constant and oscillating high glucose levels on oxidative stress, adhesion molecules expression and apoptosis in endothelial cells (HUVECs).
Materials and methods: HUVECs were incubated for 14 days in either low (5 mmol/L) or high (20 mmol/L) glucose concentrations, or to oscillating high and low glucose on a daily basis. The effect of rosuvastatin (1 µmol/L) in the presence or absence of mevalonate (200 µmol/L) was evaluated.
Results: Constant high glucose levels increased p47-phox, p67-phox and p22-phox expression (components of NADPH), eNOS, NO and O_2^- production, nitrotyrosine, 8-OHdG, ICAM-1, VCAM-1 and E-Selectin, Caspase-3 expression, and reduced Bcl-2 expression. These effects were also significantly increased in oscillating high glucose conditions. Rosuvastatin, both in constant and oscillating glucose conditions, normalized all these parameters, a beneficial effect which was abolished by mevalonate. These data suggest that rosuvastatin prevented apoptosis of HUVECs induced by high glucose exposure, reducing oxidative stress, as demonstrated by the effect on NO, superoxide, nitrotyrosine and 8-OHdG. The antioxidant action of rosuvastatin is related to the inhibition of components of NADPH oxidase over-expression in high glucose. Normalizing of eNOS expression, in the presence of increased production of superoxide, may lead to an over-generation of peroxynitrite, as demonstrated by the increase of nitrotyrosine. The beneficial effect of rosuvastatin is related to the inhibitory activity on HMG-CoA reductase, since mevalonate supplementation abolishes these effects.
Conclusions: Thus rosuvastatin may have utility in protecting the vascular endothelium form high glucose exposure in conditions such as metabolic syndrome and diabetes.

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Experimental immunology

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Intensive insulin therapy prevents diabetes and reverts protein expression changes in syngeneic islet transplants in diabetes prone BioBreeding rats

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Background and Aims: In type 1 diabetes the insulin-producing β-cells in the islets of Langerhans are selectively destroyed during complex interactions between various environmental factors and immune mechanisms in genetically susceptible individuals. Prophylactic subcutaneous insulin treatment reduces diabetes incidence and insulitis in spontaneous diabetic mice and rats. Interleukin-1β (IL-1β) is involved in β-cell destruction and induces changes in the expression of 82 proteins in isolated BB-DP rat islets *in vitro*. Our aim was to evaluate the effect of prophylactic insulin treatment *in vivo* during development of diabetes, on proteins known to change expression in BB-DP rat islets exposed to IL-1β *in vitro*.
Materials and Methods: Neonatal BB-DP rat islets were isolated by collagenase digestion and transplanted (200) to 30 day old BB-DP rats under the kidney capsule. Rats were randomised to either continuous insulin (Linplant, n=66, 1 unit of bovine insulin per day for the first 40 days and 2 units per day from day 40 and onwards) or placebo (Linplant Blank, n=55) treatment. This insulin treatment reduced the diabetes incidence from 65% to 19%. Rats were weighed and blood glucose (BG) was measured prior to transplantation and three times weekly. Rats were sacrificed 7, 23, 50, 90 days after transplantation or at onset of diabetes (BG's above 14 mmol/L). The transplants were dissected free of the kidney and metabolically labelled *in vitro* with S^{35}-methionine for 20 hours before two dimensional gel electrophoresis (2-DGE). The images of the 2-DG's were analysed and aligned and the 82 IL-1β influenced protein spots identified *in vitro* were re-localised in the transplants and followed during development of diabetes and protection from diabetes by prophylactic insulin treatment. A change in expression was considered significant if $p<0.05$ (Student's t-test).
Results: In total 2.135 protein spots were identified in all transplants and could be followed throughout development of diabetes (placebo) and during prophylactic insulin treatment from day 7 until day 90 after transplantation or onset of diabetes (n=5–6 in each group at each time point). All 82 proteins could be re-localized in all transplants and followed. Of these, 50 proteins changed level of expression and 32 proteins did not change at any point in time. In the insulin treated group, several proteins involved in signal transduction, differentiation and apoptosis were changed when compared to placebo treated animals. IL-1β effects *in vitro* on e. g. Galectin-3, Rho GDP dissociation inhibitor, Eukaryotic initiation factor 4A and Dihydropyrimidinase related protein-2 (TOAD 64) were all reversed by insulin treatment. In animals in the insulin and placebo treated groups that developed diabetes the protein expression patterns were similar and only two of the 82 spots were differently expressed (both tubulin beta-5 chain and up-regulated by insulin treatment).
Conclusion: These findings support that changes in expression of islet proteins, induced by IL-1β *in vitro*, are reversed in response to intensive treatment with insulin, suggesting that IL-1β induced protein changes seen *in vitro* also may play a role during diabetes development in the BB-DP rat *in vivo*.
Support: Danish Diabetes Association, Michaelsen Foundation, University of Copenhagen, Novo Nordisk A/S and JDRF

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Induction of regulatory T cells by vaccination with tolerogenic dendritic cells

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Background and Aims: Type 1 diabetes results from an autoimmune destruction of beta-cells. A promising method to modulate the autoimmune response is the vaccination with tolerogenic dendritic cells (DCs). The aim of this study was to analyse the mechanisms of tolerance induction after immunisation of NOD mice with insulin-pulsed DCs.
Materials and Methods: 8-weeks-old female NOD mice were treated with cyclophosphamide (200 mg/kg) to synchronise diabetes development. At day 2, mice were immunised with a single i.p. injection of 4×10^5 insulin-pulsed or ovalbumin-pulsed myeloid DCs. The T cell response was analysed by proliferation assay and the measurement of antigen-stimulated cytokine secretion (ELISpot Assay, ELISA). In addition, T cells from diabetic mice were cotransferred with CD4+ T cells from immunised mice into NOD-scid mice.
Results: The incidence of diabetes was significantly reduced by an immunisation with insulin-loaded DCs (20%) as compared to controls (80%). Protection from diabetes was associated with a significant reduction in IFN-gamma spot-forming units and an increase in IL-10 secretion ($p<0.01$). CD4+ T cells from DC-insulin immunised mice actively suppressed diabetes transfer in NOD-scid recipients (17% versus 100% diabetes).
Conclusion: Vaccination with DCs can induce an antigen-specific tolerance. Our data demonstrate that protection from diabetes is at least in part mediated by the activation of regulatory T cells. Vaccination with DCs is a useful tool for antigen-specific immunomodulation and may represent a novel approach to prevent type 1 diabetes.

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Toll-like receptor 4 controls the development of autoimmune diabetes in mice

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Background and Aims: In mammalians toll-like receptors (tlrs) are crucial for the primary recognition of microbial antigens and for the induction of immune responses against these structures. Recent reports implicate a role of tlrs also in the induction of immune reactivity against autologous antigens and in the pathogenesis of autoimmune disorders. In our study we investigated the effect of tlr4 on the progression of immune reactivity against autologous pancreatic beta cells and on the development of autoimmune diabetes in mice.
Materials and Methods: Our studies are based on the model of the tlr4 deficient mouse strain C57BL10/ScCr carrying a tlr4 defect due to a spontaneous deletion of the tlr4 encoding DNA region (tlr4–/–). The tlr4 defect of this mouse strain was backcrossed on the background of the NOD mouse for more than 8 generations and by intercrossing heterozygous littermates from the backcross generations tlr4+/+, tlr4+/– and tlr4–/– mice were generated.
Results: After more than 8 generations of backcrossing successful introduction of the tlr4 defect onto the NOD background was confirmed by demonstrating homozygosity of the diabetes associated alleles of the loci idd 1, 2, 3 and 4. As a functional proof for the presence of the tlr4 defect macrophage-enriched spleen cell populations were exposed to the tlr4 ligand lipopolysaccharide (LPS, 10 ng/ml, 6 h) and the TNFα accumulation was determined by ELISA. LPS-exposed cell populations of tlr4+/+ and tlr4+/– mice produced 225 ± 122 pg/ml and 271 ± 102 pg/ml TNFα whereas cell populations of tlr4–/– mice produced only 9 ± 4 pg/ml TNFα ($p<0.001$). When monitoring the diabetes development in the group of female tlr4+/+ mice the first case of diabetes (BG>250 mg/dl) was observed at 148 d of age and until 210 d of age 71% of the mice developed diabetes (mean age of manifestation 177 ± 22 d). Interestingly, female tlr4–/– and tlr4+/– mice showed a significant acceleration of diabetes development. In tlr4–/– and tlr4+/– mice first disease manifestations were observed at 99 and 91 d. In tlr4-/– mice the diabetes incidence reached its maximum (80%) already at 146 d (mean age of manifestation 118 ± 21 d, $p<0.01$ compared to tlr4+/+). Tlr4+/– mice showed a diabetes incidence of 67% with an intermediate mean age of diabetes manifestation of 129 ± 40 d ($p<0.05$ compared to tlr4+/+). Histological analysis at d 100 showed various stages of immune cell infiltration in the islets of tlr4+/+ mice. Interestingly, pancreata of tlr4–/– and tlr4+/– mice showed significantly increased proportions of islets ($p<0.05$) with pronounced mononuclear infiltration and advanced stages of severe intra-insulitis. In many cases inflammation remained not restricted to the islet area but extended into large areas of the surrounding exocrine tissue. When isolated islet cells of tlr4+/+ and tlr4–/– mice were exposed to a mixture of 50 U/ml TNFα, 5 U/ml IL-1β and 10 U/ml IFNγ (6 d) 46 ± 5% of tlr4+/+ and 42 ± 7% of tlr4–/– islet cells were lysed. This finding demonstrates that the status of tlr4 expression does not affect islet cell sensitivity towards the damaging effects of inflammatory cytokines.
Conclusion: Our data obtained with tlr4 deficient NOD mice show that tlr4 controls the inflammatory pathways involved in beta cell destruction and in the development of autoimmune diabetes. Tlr4 does not affect beta cell susceptibility to inflammatory damage but seems to act on immunoregulatory processes that may involve the activity of regulatory T-cells (Treg).

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Characterization of primary and immortalised human pancreatic islet endothelial cells

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Background and Aims: Intra-islet microvascular endothelial cells (MEC) are likely to play a pivotal role in islet physiology and the pathogenesis of types 1 and 2 diabetes. It is known that islet MECs deliver oxygen and nutrients to endocrine cells, induce insulin gene expression during islet development, and not only facilitate rapid release of insulin, but also appear to have a role in fine-tuning blood glucose sensing and regulation. Moreover, studies support the possibility that the islet microvasculature participates in sensing the environment of the islets and generating signals to induce the growth and maintenance of the islets in response to physiological stimuli. Further, human and murine studies indicate that during autoimmune insulitis in type 1 diabetes the islet MECs adopt an activated phenotype and are likely to be involved in regulating mononuclear cell accumulation in the islets. Studies on the biology of MECs surrounding and penetrating the pancreatic islets are hampered by difficulties in isolating and culturing large numbers of pure cells. Against this background, in the present study, we aimed to isolate, purify and characterise human islet MECs and to establish an immortalised cell line.
Materials and Methods: Human islet MECs were extracted and purified using anti-CD105 coated immunomagnetic beads, analysed by scanning electron microscopy, and endothelial markers and surface molecules analysed by flow cytometric analysis. Surface molecules were also analysed after TNF-α and IFN-γ stimulation. The expression of nephrin, a highly specific barrier protein, was assessed by IF, flow cytometry, and WB on cell lysates. Mononuclear cell adhesion and transwell migration assays were also performed. An immortalised cell line was then established by using a chimeric adeno5/SV40 virus, and comparative analyses performed.
Results: Islet MECs expressed the classical endothelial markers, basal high level of the adhesion molecule ICAM-1, low level of E-selectin and TNF-α inducible VCAM-1. IFN-γ induced expression of HLA-DR class II molecules. The immortalised islet MECs expanded rapidly, exhibited an increased DNA synthesis, and were passaged approximately 30 times, without signs of senescence. The immortalised islet MECs retained the endothelial characteristics of the parental cells, without signs of tumorigenic transformation. Moreover, they behaved as the primary cells in terms of TNF-α stimulation of expression of adhesion molecules and support of leukocyte adhesion and transmigration. Both primary and immortalised islet MECs expressed nephrin.
Conclusion: Islet MECs exhibit distinctive morphological characteristics. The immortalised microendothelium represents a pancreatic islet EC line capable of growth and stable phenotype. The immortalised islet MECs we have established could therefore effectively represent a substitute of their primary counterparts for *in vitro* studies on the role of microvasculature in the pathophysiological processes involved in type 1 and type 2 diabetes.

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Functional characterisation of T-cells of the T1DM model LEW.1AR1-iddm

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Background and Aims: The LEW.1AR1-iddm rat, an animal model of human type 1 diabetes mellitus (T1DM), originated through a spontaneous mutation within the congenic inbred strain LEW.1AR1. Adoptive transfer of lymphocytes has shown that both autoaggressive and protective cells can be transferred. Recently, flow cytometry of peripheral blood lymphocytes (PBL) indicated that the T-cell content of LEW.1AR1-iddm rats is altered as compared to the background strain LEW.1AR1. The aim of the study was (a) to characterise the T-cell subpopulation that is capable to induce or to prevent T1DM and (b) to screen the T-cell repertoire of PBLs in LEW.1AR1-iddm rats during a lifelong study.
Materials and Methods: T-cells were isolated by MACS separation using monoclonal antibodies (rat CD4 Microbeads, rat anti-mouse IgG1 Microbeads, Miltenyi; 341 purified, BD). Then (a) CD4+ or CD8+ T-cells from diabetic LEW.1AR1-iddm rats were selectively transferred into athymic LEW.1AR1-Whnrnu rats and (b) CD4+ or CD8+ T-cells from LEW.1AR1 rats were transferred into pre-diabetic LEW.1AR1-iddm rats. PBLs were differentiated by flow cytometry using a set of monoclonal antibodies: OX-38 FITC, OX-8 FITC, OX-8 PE, G4.18 PE, R73 FITC, OX-33 FITC, NKR 10/78 FITC, all BD; ED1 FITC, Serotec.
Results: After adoptive transfer of CD4+ T-cells from diabetic LEW.1AR1-iddm rats into athymic LEW.1AR1-Whnrnu rats 60% of the recipients developed diabetes while CD8+ T-cells were not able to induce T1DM. On the other hand, CD4+ T-cells adoptively transferred from T1DM-resistant LEW.1AR1 rats into pre-diabetic LEW.1AR1-iddm rats could not prevented T1DM development. Alterations of the T-cell content in the course of life of LEW.1AR1-iddm rats had an effect on the CD4+/CD8+ T-cell ratio, which varied from 0.9 and 2.2. The CD4/CD8 T-cell ratio in PBLs of the background strain LEW.1AR1 was 2.1 ± 0.1.
Conclusion: Recent investigations in diabetic patients indicate that alterations in the leukocyte repertoire may play a roll in the pathogenesis of T1DM. Thus, alterations of the CD4+/CD8+ T-cell ratio in LEW.1AR1-iddm rats may be involved in the development of T1DM. Moreover, it became evident that either the CD4+T-cell population or a subpopulation of CD4+ T-cells can elicit T1DM in athymic recipients. Although CD4+CD25+ T-cells are known as regulatory T-cells processing a protective potential, the plain CD4+ T-cell population from T1DM resistant LEW.1AR1 rats was not able to prevent diabetes development in LEW.1AR1-iddm rats. Thus, the LEW.1AR1-iddm model and its coisogenic LEW.1AR1 background strain provide a valuable model system to elucidate the role of T-cells in T1DM.

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Experimental autoimmune diabetes (EAD) - a new animal model to study and modify beta cell autoimmunity

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Background and Aims: Insulin has been used to modulate T cell dependent autoimmunity in type 1 diabetes (T1D). We have previously shown that insulin can adversely induce diabetes in RIP-B7.1 (CD80) transgenic mice. Based on these findings we have developed EAD as a novel animal model of T1D.
Materials and Methods: For in vivo DNA vaccination studies in B7.1 transgenic mice, plasmid vectors expressing preproinsulin (PPI) or PPI domains were constructed. T cell activation and cytokine profiles were analyzed by ELISPOT and FACS staining to identify disease-associated T cell epitopes. To define the role of interferon-gamma (IFNg) in EAD, IFNg-deficient B7.1 transgenic mice were generated. T cell subsets were isolated by MACS for adoptive diabetes transfer studies.
Results: Hyperglycemia (>250 mg/dl), CD4+/CD8+ insulitis and insulin deficiency rapidly developed after DNA vaccination with PPI or insulin in B7.1 mice (incidence 95%, median onset 3 weeks), but not IFNg-/- B7.1 mice, indicating that IFNg is critical for diabetes development in EAD. Adoptive transfer of spleen cells or CD8+ (but not CD4+) T cells from vaccinated B7.1 was followed by autoimmune diabetes in naïve syngeneic recipients. Similarly, in vivo CD8+ T cell depletion resulted in a significant delay of diabetes in EAD, while CD4+-depletion was without effect. Using PPI minigene DNA constructs we have mapped the immunogenic domains to the B and A chains of insulin, with a candidate MHC class I T cell epitope (SLYQLENYC) identified in the A chain (A12-20).
Conclusions: EAD is a new mouse model of human T1D, characterized by a central role of insulin-specific CD8+ T cell reactivity. EAD is suitable for the pre-clinical assessment of novel strategies to prevent T1D, including the therapeutic use of altered insulin peptide ligands.
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Treatment of the diabetic foot

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C-peptide improves sensory nerve function in type 1 diabetes and neuropathy

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Background and Aims: Experimental studies in animal models of neuropathy in type 1 diabetes have indicated that C-peptide replacement results in amelioration of diabetes-induced functional and structural changes in peripheral nerves. These effects may be mediated via C-peptide's stimulatory action on eNOS and Na^+,K^+-ATPase. This study was undertaken to examine if C-peptide administration in type 1 diabetes patients with neuropathy improves sensory nerve function.
Materials and Methods: The study was a 6 mo double-blind, randomized, placebo-controlled study, with 3 study arms. All patients had type 1 diabetes and neuropathy as defined by the San Antonio conference consensus criteria. C-peptide was given at two dose levels (500 and 1,500 nmol/24 h in 4 s.c. doses). The study involved 5 centers in Sweden; 526 patients were screened, 161 randomized and 139 completed the study. Mean age of the patients was 43 ± 0.1 yrs and their diabetes duration was 29.4 ± 0.1 yrs. 40% reported known peripheral neuropathy and 35% subjective symptoms. Neurological, neurophysiological measurements and quantitative sensory testing (QST) were performed before and after 6 mo treatment. Clinical neurological impairment was assessed using a composite score (NIA score) based on sensibility evaluation at the big toe, the dorsum of the foot and the lower leg plus determination of reflexes; 86% of the patients had a pathological NIA score at baseline.
Results: Sensory nerve conduction velocity (SCV) was 2.6 ± 0.05 SD below the body height-corrected normal value at baseline and improved by 0.48 ± 0.19 m/s in the C-peptide groups (P<0.007). The low and high C-peptide dose gave similar results. In the least severely diseased patients (SCV <2.5 SD below normal at baseline, n=70) SCV improved by 1.0 m/s (P<0.014 vs. placebo). The NIA score improved after C-peptide treatment (P<0.012 within the group) and vibration perception at the foot and the lower leg tended to improve. HbA_{1c} levels were unchanged throughout the study. These results confirm and extend the previously reported C-peptide-induced improvement in patients with subclinical neuropathy.
Conclusion: C-peptide treatment for 6 mo in type 1 diabetes patients with early stage neuropathy significantly improves sensory nerve function.

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Long-term euglycemia after pancreas transplant alone (PTA) normalizes somatic and autonomic peripheral nerve function (PNF) in type 1 diabetic patients (DM1).

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Background and Aims: The effects of PTA on PNF of DM1, both on somatic and autonomic components, are still debated. Aim of this study was to evaluate the changes induced by PTA both on symptoms and signs of PNF in DM1 patients after 2 years of continuous normo-glycemia.
Materials and Methods: We prospectively studied 11 out of 56 consecutive successful PTA recipients [Age 31.5 ± 3.5 yrs, Duration of Diabetes (DD) 20.0 ± 4.9 yrs] who reached 2-year follow-up with sustained euglycemia, by scoring symptoms by means of Michigan Neuropathy Screening Inventory (MNSI – score) and assessing PNF with vibration perception threshold (VPT – volts), lying-to-standing test (LS – 30/15 ratio), conduction velocity (NCV – m/s) and potential amplitude (PA – mv) of deep peroneal nerve. Results were compared with those obtained from 10 intensively treated DM1 [Age 31.5 ± 3.5 yrs, DD 20.0 ± 4.9 yrs] and 12 age-matched non-diabetic Controls (C).
Results: MNSI improved in PTA (1.1 ± 0.4 vs 3.1 ± 0.9, p<0.05) and worsened in DM1 (4.2 ± 1.3 vs 2.0 ± 1.1, p< 0.05). Indexes of PNF (Table) unanimously normalized in PTA and deteriorated further in DM1.

	Baseline	+ 24 months
VPT PTA	15.0 ± 10.7*	7.4 ± 3.6#
VPT DM1	17.3 ± 8.8*	20.2 ± 7.4#*
VPT C	8.1 ± 4.1	10.2 ± 5.5
LS PTA	1.3 ± 0.6*	2.3 ± 0.7#
LS DM1	1.4 ± 0.8*	1.2 ± 0.5#*
LS C	2.2 ± 0.9	2.0 ± 0.6#
NCV PTA	43.1 ± 3.9*	46.5 ± 3.8#
NCV DM1	44.3 ± 3.7*	41.6 ± 3.2#*
NCV C	46.7 ± 4.0	45.4 ± 3.9
PA PTA	2.4 ± 1.2*	3.6 ± 1.4#
PA DM1	3.5 ± 1.9	2.5 ± 1.3#*
PA C	3.8 ± 1.4	3.5 ± 1.6
HbA1c – PTA (%)	8.2 ± 1.8	5.5 ± 0.4#*
HbA1c – DM1 (%)	8.4 ± 1.9	7.5 ± 1.6#

#p<0.05 or less vs baseline; *p<0.05 or less vs C

Conclusion: Successful PTA, by means of long-term stable euglycemia, normalizes both autonomic and somatic PNF, at variance with intensively-treated DM1.

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Glyceryl trinitrate spray in the management of painful diabetic neuropathy: a randomized double blind placebo controlled cross over study

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Background and Aims: Various drugs are effective in the management of painful diabetic neuropathy, but none is completely satisfactory. To test the effectiveness and safety aspect of glyceryl trinitrate in the management of painful diabetic neuropathy as a Nitric Oxide (NO) donor with local vasodilating properties in spray form.
Materials and Methods: Randomized double blind placebo controlled cross over study.
50 consecutive patients with type 2 diabetes mellitus with painful neuropathy were asked to participate in the trial: 50 agreed. Two were excluded: (1 with HbA_1c >11 and one who withdrew consent). The remaining 48 were given either drug (group A) or placebo (group B) in first phase. After thorough clinical assessment in the first phase, quantitative assessment of pain was done by McGill Pain Questionnaire, Visual analogue Score, Present Pain Intensity and 11 point Lickert scale, at the beginning, after 4 weeks, followed by 2 weeks wash out period and there after 4 weeks of receiving crossover regimen. Adverse drug effects were assessed periodically.
Results: Of the 48 patients, five dropped out, two in group A and three in group B. Both groups A and B experienced significant improvement in pain score in their drug phase of trial, when compared to placebo phase of other group (p<0.001). After crossing over the treatment arm, patient observed significant improvement in all pain score compared to group A (p<0.001). The numbers needed to treat (NNT) calculated on VAS as pain parameters came out to be 4. The drug was well tolerated by all patients except palpitation and headache for some days in 5 patients.
Conclusion: GTN spray, a well tolerated drug provides significant improvement in painful diabetic neuropathy. These data provides a basis for future trials of longer duration in a larger group of patients.

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The SIDESTEP study of diabetic foot infections (DFI): a multicenter, double-blinded, randomized, controlled trial (RCT) of ertapenem (E) vs. piperacillin/tazobactam (P/T)

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Background and Aims: DFI are common and serious, but few RCTs have compared efficacy of different antibiotic regimens for these frequently polymicrobial infections. Most previous studies were small and not masked.
Materials and Methods: We compared intravenous (IV) E (1 g/d), a group 1 carbapenem, and P/T (3.375 g qid) for treatment of patients with moderate to severe DFI in a double-masked study designed to determine noninferi-

ority of E to P/T for DFI. IV antibiotic therapy was required for a minimum of 5 d. Patients could be switched to oral therapy (amoxicillin/clavulanate) for a maximum cumulative treatment of 28 d. Baseline and follow-up visits included evaluation of tissue wound cultures, quantitative wound scores, digital photography, dermal thermography, and hematological studies. The primary endpoint was the % of patients with a favorable clinical response (cure or improvement) at the visit during which IV antibiotic was discontinued (DCIV). Additional assessments were made at 10 d of follow-up.
Results: 576 patients were randomized to treatment (E: 289; P/T:287) and 445 were evaluable at the end of IV therapy (E: 226; P/T: 219). Clinical success rates were similar between the treatments at DCIV (E: 94.2%, P/T: 92.2%; between treatment difference: 1.9; 95% CI: -2.9, 6.9). Similar clinical success rates were also observed in the 402 patients evaluable at 10-day follow-up assessment (E: 87.4%, P/T: 82.7%; between treatment difference: 4.7; 95% CI: -2.2, 11.9). During parenteral therapy, there was no difference in incidence of drug related clinical adverse experiences (E: 15.2%, P/T: 19.9%) or in serious clinical drug related AEs (E: 0.3%, P/T: 0.3%).
Conclusion: This study, the largest, most comprehensive and only double-blinded RCT of antibiotics for DFI, found that clinical and microbiological outcomes for patients treated with E once daily were equivalent to that of patients treated with P/T q 6 h.

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Intranasal calcitonin in the treatment of acute Charcot neuroosteoarthropathy including patients with renal insufficiency: a randomized controlled trial

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Background and Aims: Excess osteoclastic activity is believed to be responsible for the bone destruction in Charcot neuroosteoarthropathy (CNO). In contrast to bisphosphonates, we have no clear data about intranasal calcitonin treatment of acute CNO. Intranasal calcitonin should be preferred in patients with renal insufficiency, which is frequent in pacients with CNO. The purpose of our study was to evaluate the effect of intranasal calcitonin in the treatment of acute CNO in a randomized controlled trial.
Materials and Methods: 28 patients (mean age 51.6 ± 12.3 years, 64% Type 2 diabetes) with acute CNO treated in our foot clinic during the period 8/2003–12/2004 were randomized to receive intranasal calcitonin 200 IU/day together with calcium supplementation 1000 mg/day (Group A – 15 patients) or calcium supplementation 1000 mg/day in monotherapy (Group B – 13 patients); all patients also had standard treatment of the CNO. Skin temperatures and markers of bone turnover (bone specific alkaline phosphatase- bALP, carboxy-terminal telopeptide region of type I collagen-ICTP, osteocalcin) were measured over the 6 months, in 5 visits. Renal insufficiency was recorded in 9 patients (Group A – 5 patients, Group B – 4 patients), no patient required haemodialysis.
Results: Before treatment there was no significantly differences between group A and B in the mean skin temperature difference 3.4 ± 1.3 °C vs. 3.5 ± 1.6 °C, bALP 16.51 ± 8.55 vs. 15.73 ± 7.1 ug/l, ICTP 10.16 ± 4.20 vs. 10.09 ± 3.58 ug/l, osteocalcin 6.41 ± 3.68 vs. 7.59 ± 5.44 ng/ml. There was a significantly fall in ICTP in group A at 1, 2, 3, 6 month of follow up ($p<0.01$, $p<0.001$, $p<0.001$, $p<0.01$), but in group B only at 6 month ($p<0.05$). In addition there was significantly greater reduction in ICTP in group A during 3 months in comparison with group B ($p<0.05$). Skin temperature and bALP significantly decreased in both groups during the whole follow-up period, mostly at 3 month ($p<0.001$, $p<0.05$), but no difference was seen between groups. The level of osteocalcin significantly decreased in both groups during the whole follow-up period ($p<0.05$), but without significantly difference between both groups.
Conclusion: For the first time it was demonstrated that in addition to standard therapy intranasal calcitonin treatment together with calcium supplementation produced significantly greater decreases in bone turnover than calcium supplementation without calcitonin in the treatment of active Charcot neuroarthropathy. Intranasal calcitonin, in contrast to bisphosphonates, should also be used in patients with renal insufficiency.
This study was supported by the IKEM (MZO 00023001).

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Duloxetine in the treatment of diabetic peripheral neuropathic pain – results from three clinical trials

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Background and Aims: The efficacy and safety of duloxetine, a dual reuptake inhibitor of serotonin and norepinephrine, on the treatment of diabetic peripheral neuropathic pain (DPNP) was assessed in 3 studies.
Materials and Methods: Patients with DPNP of at least 6 months duration, and without depression, were enrolled in the 12-week acute therapy studies. Study 1 (N=457) had treatment groups of duloxetine 20-mg once daily (QD), 60-mg QD, 60-mg twice daily (BID), and placebo; Studies 2 (N=334) and 3 (N=348) compared duloxetine 60-mg QD and 60-mg BID with placebo. The primary outcome measure was the weekly mean score for 24-hour average pain severity based on an 11-point Likert scale.
Results: Across all three studies, duloxetine 60-mg QD and 60-mg BID demonstrated significant treatment effect on DPNP and showed rapid onset of action, with separation from placebo occurring at week one on the 24-hour average pain severity score ($p<0.001$). This finding was confirmed in most secondary measures for pain. Duloxetine 60-mg QD and 60-mg BID achieved similar efficacy results on most measures, with duloxetine 60-mg BID showing significantly more improvement on some McGill pain descriptors. The evaluation of Clinical Global Impression of Severity and Patient Global Impression of Improvement demonstrated superiority of duloxetine 60-mg QD and 60-mg BID over placebo. A significant treatment effect for duloxetine was observed for most health outcome measures. Duloxetine showed no adverse effects on diabetic control or complications, and was safely administered and well tolerated.
Conclusion: In these clinical trials, duloxetine (only FDA-approved drug for DPNP) was an efficacious and safe treatment for DPNP.
Support: Eli Lilly and Company

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Prediction of cardiovascular disease

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Low serum adiponectin as a predictor of coronary heart disease. A population-based, 10-year follow-up study in elderly men

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Background and Aims: In cross-sectional studies a relationship between adiponectin, obesity and coronary heart disease (CHD) have been reported. In this study we have investigated the longitudinal association between adiponectin and CHD in a population-based cohort of elderly men.
Materials and Methods: The subjects participated in the Uppsala Longitudinal Study of Adult Men. A baseline investigation was carried out at age 70 from August 1991 to May 1995 in 918 men with a follow-up of up to 10 years using Swedish national registry data. The baseline investigation included anthropometry, OGTT, blood pressure, smoking, serum lipids, a euglycaemic insulin clamp and fasting serum adiponectin, which was analysed blinded for outcome, using a validated in-house time-resolved immunofluorometric assay. The main outcome measure, CHD, was defined as death, as recorded in the Cause of Death Registry, or first time hospitalised for CHD as recorded in the In-Patient Registry both kept at the National Board of Health and Welfare, Sweden. CHD was defined according to ICD 9 codes 410 to 414. Associations were analyzed using Cox's proportional hazards regression, presented as hazard ratios (HRs) with 95% confidence intervals (CIs) for a one SD increase in the predictor variable.
Results: In a multivariate analysis including total cholesterol (HR, 1.25, CI, 1.06–1.49), smoking (HR, 1.62, CI, 1.11–2.36) and systolic blood pressure (HR, 1.28, CI, 1.09–1.51) serum adiponectin was associated with lower risk of CHD (HR, 0.77, CI, 0.65–0.92. The association was independent of BMI but was weaker with adjustment for insulin-mediated glucose disposal.
Conclusion: Even after adjustment for known cardiovascular risk factors serum adiponectin was associated with a lower risk of CHD in a longitudinal study.

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Alanine aminotransferase is a predictor of coronary heart disease events: the Hoorn Study

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Background and Aims: Several studies have demonstrated that even slightly elevated alanine aminotransferase (ALT) is a predictor of incident type 2 diabetes mellitus (DM2). Since we recently observed an association between ALT and endothelial dysfunction in uncomplicated DM2 subjects, the aim of the present study was to assess the association between ALT and incident coronary heart disease (CHD) in a population-based study of glucose tolerance and related complications in Caucasian men and women aged 50 to 75 years.
Materials and Methods: In the Hoorn Study we assessed the ten-year risk of fatal and non-fatal coronary events in relation to ALT enzyme activity in 1578 subjects, with Cox survival analysis. Subjects with DM2, prevalent CHD and missing data on outcome and/or confounding variables at baseline, were excluded.
Results: The incidence of coronary events was 1.39 per 100 person-years. The relative risk (hazard ratio) of fatal and non-fatal coronary events was 1.49 (95CI%: 1.07–2.06) for subjects in the upper tertile compared to those in the first and second tertile. This risk was independent of age, sex, alcohol-intake, smoking, physical activity, systolic and diastolic blood pressure, triglycerides, HDL-cholesterol, waist and HbA1c. Adjustment for fasting plasma glucose concentrations and/or 2-hour post-load plasma glucose concentrations did not substantially change the presented associations.

ALT and CHD events (hazard ration; 95%CI) (n=1578)

Model	ALT (3rd versus 1st and 2nd tertile) HR (95% CI)	P-value
Non-fatal CHD events (n = 161)		
Model 1	1.53 (1.10–2.12)	0.011
Model 2	1.46 (1.03–2.06)	0.031
Fatal CHD events (n = 21)		
Model 1	1.25 (0.55–3.09)	0.63
Model 2	1.11 (0.41–3.04)	0.84
Fatal and non-fatal CHD events (n = 174)		
Model 1	1.56 (1.14–2.13)	0.005
Model 2	1.49 (1.07–2.06)	0.017

Model 1 adjusted for age and sex; Model 2 adjusted for age, sex, alcohol-intake, cigarette smoking, physical activity, triglycerides, HDL-cholesterol, systolic and diastolic blood pressure, waist and HbA1c

Conclusion: These data suggest that ALT is associated with coronary events independent of traditional risk factors, including impaired glucose metabolism. Further studies are warranted to confirm these findings and to elucidate the pathophysiological mechanisms.

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Gender differences in the prediction of acute coronary events and stroke by fasting plasma glucose and OGTT

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Background and Aims: The aim of the present analysis was to assess possible gender differences in the prediction of fasting blood glucose (f-glu) and 2-h glucose (2 h-glu) post oral glucose tolerance test (OGTT) in self-reported non-diabetic, healthy subjects for the risk of acute coronary events (ACE) and stroke (fatal and non-fatal).
Materials and Methods: We analyzed data from the Malmö Preventive Project, a population-based screening project designed to identify risk factors for cardiovascular disease (CVD). In the current set of analyses, we focused on 18,932 subjects (13,046 men; 5,886 women) aged 26–62 years (mean: 49.6, SD 4.9), who were free of known diabetes and previous CVD, and underwent an OGTT (75 g glucose) at baseline. Data on medical history, smoking, body mass index (BMI), blood pressure, fasting and 2-h blood glucose levels, triglycerides, and total cholesterol were obtained at baseline. The number of ACE (n= 2,310) and stroke (n= 1,178) was identified from Swedish national registers. Mean follow-up time was 19.1 (SD 6.3) years. Hazard ratio (HR) for incident CVD was estimated using Cox regression analysis, stratifying by gender, and adjusting for age, body mass index, and additionally for other risk factors.
Results: In both men (M) and women (W), f-glu was a stronger predictor of CVD [HR: M 1.08 (95%CI:1.05–1.12); HR: W 1.13 (95%CI: 1.05–1.21)] than 2 h-glu [HR: M 1.03 (95%CI:1.00–1.07); HR: W 1.10 (95%CI: 1.00–1.20)]. After subdividing CVD into ACE and stroke events we found that f-glu is still predictive for ACE in both genders [HR: M 1.07 (95%CI:1.03–1.12); HR: W 1.17 (95%CI: 1.07–1.27)]. For women, but not for men, 2 h-glu was also an independent predictor of ACE [HR: W 1.18 (95%CI: 1.06–1.32)]. In men, only f-glu was a significant predictor for stroke [HR: M 1.13(95%CI: 1.05–1.20)]. Additionally, adjusting for systolic blood pressure, total cholesterol, triglycerides, and smoking did not significantly affect these results.
Conclusion: On overall, fasting blood glucose level seems to provide a more accurate prediction of cardiovascular events compared to post-OGTT blood glucose in middle-aged men and women. Fasting glucose predicted stroke events in men only, but acute coronary events in both genders. Post-OGTT glucose level predicted acute coronary events in women, but not in men.

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The impact of diabetes and stroke at baseline and during follow-up on stroke mortality

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Background and Aims: Whether history of myocardial infarction has the same risk on coronary heart disease death as history of type 2 diabetes has been assessed in recent years. However, whether diabetes has the same risk on stroke death as incident stroke has not been studied. The aim of this study is to compare the magnitude of incident diabetes and stroke at baseline and during follow-up on stroke mortality.
Materials and Methods: We prospectively followed 25287 Finnish men and 26537 women aged 25–74 years. Data on the occurrence of incident diabetes and stroke at baseline and during follow-up, and stroke death were obtained from different registers through computerized register linkage.
Result: During a mean follow-up of 18.9 years, 1043 stroke deaths were recorded. In the baseline study, the multivariate-adjusted (age, body mass index, systolic blood pressure, total cholesterol, education, alcohol consumption, physical activity and smoking) hazard ratios for stroke mortality were 2.68 (95% CI 1.89–3.80) for men with prior diabetes only, 2.28 (95% CI 1.48–3.50) for men with prior stroke only, and 6.56 (95% CI 3.03–14.2) for men with both prior diabetes and stroke compared with men without diabetes and stroke. In women, the corresponding hazard ratios were 3.49 (95% CI 2.44–5.00), 2.60 (95% CI 1.63–4.15), and 2.38 (95% CI 0.58–9.68), respectively. When the diabetes and stroke status during the follow-up was included in the analyses, the multivariate-adjusted hazard ratios for stroke mortality were 1.41 for men and 1.56 for women with incident diabetes only, 5.62 for men and 5.60 for women with incident stroke only, and 5.59 for men and 4.50 for women with both incident diabetes and stroke compared with men and women without diabetes and stroke.
Conclusion: Diabetes and stroke, both present at baseline and during the follow-up, increase the risk of stroke death markedly. Incident stroke during follow-up has a greater risk of stroke mortality than incident diabetes.
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Weight regulation and obesity

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Orexigenic hormones ghrelin and NPY are expressed in human adipose tissue and isolated adipocytes

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Background and Aims: Ghrelin controls energy balance, enhancing fat mass deposition and food intake through the activation of the hypothalamic nuclei and the promotion of (NPY neuropeptide Y). NPY is stimulated post-prandially acting as an orexigenic hormone to act centrally on appetite regulation. As part of this the gut-fat-brain axis for energy homeostasis abdominal adipose tissue may represent a depot which may utilise both ghrelin and NPY in maintaining adequate weight balance.To date no study has examined these peptides in human adipose tissue. Therefore, the aims of this study were to 1) characterise the protein expression of ghrelin and NPY in human adipose tissue 2) determine the depot-specific expression of these peptides in abdominal and gluteo-femoral fat and 3) determine whether the peptide expression was related to adipocytes or the vascular stromal fraction as a source of these peptides.
Materials and Methods: For this study, ex vivo human adipose tissue was taken from women undergoing elective surgery (age 41+/–9.49 years (mean+/–SD), body mass index (BMI) 25.6+/–4.96 kg/m^2, n=20) and Western blot analysis was used to determine NPY and ghrelin expression in human adipose tissue. Subsequent analysis compared protein expression between fat depots (abdominal subcutaneous (AbdSc) n=20; abdominal omental (Om) n=8 and thigh: n=7).
Results: Our findings showed that NPY protein was expressed in human adipose tissue with AbSc expression approximately 2 fold higher than either Om or thigh adipose tissue (AbdSc: 1.87+/-0.23, Om: 1.03+/-0.15, thigh: 1.0+/–0.29; p= 0.029 and p= 0,035 respectively). Our data also indicated depot specific alteration of ghrelin protein expression in a similar pattern with AbdSc exhibiting 2 fold and 3 fold greater expression compared with Om and thigh adipose tissue (AbdSc: 3.05+/–0.55, Om: 1.5+/–0.5 and thigh: 1.0+/–0.4; p= 0.024 and p= 0.033 respectively). protein studies determined that both ghrelin and NPY were only detected in isolated adipocytes. Furthermore ghrelin and NPY were observed to be inversely correlated with BMI in AbdSc and Om adipose tissue (ghrelin: r= 0.635, r^2= 0.40,p= 0.027; NPY: r= 0.58; r^2 =0.337, p= 0.08).
Conclusion: In summary, our present findings highlight for the first time the protein expression of both NPY and ghrelin localised to adipocytes and expressed in a depot specific manner, highest expression being noted in AbdSc fat and inversely correlated with BMI. In conclusion, this data suggests that these peptides may have a central role and function in human obesity, other than through central appetite regulation and these roles in the control of energy balance need further investigation.

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Changes in plasma total ghrelin levels after high-protein and high-fat isoenergetic meals in lean and obese women

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Background and Aims: Ghrelin is a recently discovered peptide, mainly produced by specialized cells in the gastric fundus, which was first characterized as a potent growth hormone secretagogue. It soon, though, became evident that this hormone is a powerful orexigenic signal, acting through the activation of neuropeptide Y and agouti-related protein neurons in the arcuate hypothalamic nucleus. Its preprandial rise and rapid postprandial decline have been found to play a pivotal role in meal initiation and satiety, respectively. However, little is known about the influence of meals of different composition on postprandial ghrelin concentrations. In the present study, we examined the acute effect of two isoenergetic meals, one rich in protein and one rich in fat, on plasma total ghrelin concentrations in lean and obese women.
Materials and Methods: Nine lean (BMI =22.98 ± 2.10 kg/m^2) and nine obese (BMI =37.16 ± 8.37 kg/m^2) women, strictly matched for age, were recruited. Weight, height, waist and hip circumference were measured and BMI and waist-to-hip ratio were calculated. Percentage of body fat (% body

fat) was measured using a bioimpedance analyzer. They were then fed in random order and on different days two different isoenergetic meals. One was rich in protein (composition: 18.3 g carbohydrates, 0.2 g fat, and 102.4 g protein, with an energy content of 484.4 Kcal) and the other was rich in fat (composition: 39.4 g fat, 20 g carbohydrates, and 11.8 g protein, with an energy content of 485.3 Kcal). Plasma total ghrelin levels were measured by radioimmunoassay in the fasting state, as well as 1 hour, 2 hours and 3 hours postprandially.
Results: Fasting plasma ghrelin levels were almost double in the lean compared to the obese women (707.6 ± 80.6 vs. 353.5 ± 81.0 pmol/l, respectively, P=0.001), and were significantly and inversely related to BMI (r=–0.46, P=0.01), % body fat (r=-0.50, P=0.006), waist circumference (r=–0.54, P=0.01) and waist-to-hip ratio (r=–0.45, P=0.006). After the protein-rich meal, ghrelin levels declined progressively during the first two hours in the lean subjects (1st h: 669.4 ± 282.5 pmol/l; 2nd h: 613.2 ± 158.0 pmol/l) and their minimum value was observed at the second hour (P=0.008). They returned to their initial values thereafter (3rd h: 706.6 ± 149.1 pmol/l). No significant change was observed after the protein-rich meal in the obese women (1st h: 343.1 ± 38.4 pmol/l, 2nd h: 382.9 ± 29.2 pmol/l, 3rd h: 382.6 ± 33.9 pmol/l, ANOVA for repeated measurements, P=0.65). After the fat-rich meal, ghrelin levels did not change significantly in either lean (1st h: 682.3 ± 159.8 pmol/l, 2nd h: 674.4 ± 162.1 pmol/l; 3rd h: 638.2 ± 154.9 pmol/l, P=0.39) or obese women (1st h: 430.2 ± 52.6 pmol/l, 2nd h: 430.1 ± 49.3 pmol/l; 3rd h: 404.9 ± 63.3 pmol/l, P=0.28).
Conclusion: Plasma total ghrelin levels decline significantly after consumption of a protein-rich meal in lean but not in obese women. This is consistent with the hypothesis that ghrelin-mediated satiety mechanisms are compromised in obese individuals. Moreover, consumption of a fat-rich meal does not significantly affect plasma ghrelin levels in either lean or obese subjects, a fact which suggests a possible failure of fat to induce satiety.

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Monocyte chemoattractant protein 1 (MCP-1): a possible link between visceral and adipose tissue - associated inflammation and subclinical echocardiographic abnormalities in uncomplicated obesity
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Background and aim: Obesity can be considered a state of chronic, low-grade inflammation. Particularly, visceral adipose tissue (VAT) seems to be an active compartment in the secretion of pro-inflammatory molecules. Adipocytes and VAT are able to produce great amount of the monocyte chemoattractant protein 1 (MCP-1) a chemokine directly involved in ventricular remodelling and atherosclerosis through macrophage infiltration. The aim of this study was to explore the possible existence of a correlation between MCP-1, abdominal fat accumulation and echocardiographic abnormalities in uncomplicated obesity.
Methods and methods: Plasma MCP-1, C-reactive protein levels (CRP) and echocardiographic parameters were assessed in 20 normotensive obese women (OB) in fertile age [mean 32.7 ± 9.1 years; mean body mass index (BMI), 43.9 ± 4.8 kg/m^2] and 12 sex- and age-matched healthy, normal-weight controls (C) (mean 36.8 ± 8.2 years; mean BMI, 22.6 ± 1.7 kg/m^2). VAT in the obese group was assessed by computed tomography (CT) at L4 level.
Results: The obese patients had higher plasma levels of MCP-1 than controls (OB: 66.3 ± 7.2 vs C: 40.9 ± 2.4, pg/ml, p<0.0001). MCP-1 levels were correlated with VAT area (r=0.44, p<0.002), CRP (p<0.003), end-diastolic posterior wall (PW) (p<0.03), relative wall thickness (RWT) (p<0.03), early diastolic filling wave velocity (E) (p<0,04), isovolumetric relaxation time (IVRT) (p<0.03) and deceleration time (DT) (p<0,05). VAT area was also correlated with different echocardiographic parameters: PW (p<0.03), RWT (p<0.002), E (p<0.001), E/atrial diastolic filling wave velocity (E/A ratio) (p<0.03) and myocardial performance index (MPI) (p<0.04). Female patients with greater amount of visceral fat (VAT >130 cm^2) presented higher MCP-1 and CRP levels than those with a lower degree of abdominal adiposity (VAT <130 cm^2): MCP-1, 77.3 ± 10.2 vs 48.2 ± 1.9 pg/ml, p<0.02; CRP, 1.4 ± 0.8 vs 0.6 ± 0.4 mg/dl, p<0.05. In addition, PW, RWT, DT and MPI were also significantly higher in patients with the greatest amount of VAT.
Conclusions: These data suggest that MCP-1 levels and visceral adipose tissue are associated with some morphological and functional echocardiographic abnormalities and may also support a role of visceral fat in predisposing to cardiac dysfunction, possibly through a low-grade state of inflammation. The role of VAT in MCP-1 release might emphasize the importance of macrophage infiltration in determining the low-grade inflammatory state associated with visceral obesity.

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Variations in the adiponutrin gene show association to obesity
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Background and Aims: Adiponutrin (ADPN) is a newly discovered gene located on chromosome 22q13 and encodes for a 413 amino acid membrane protein with lipase activity. The gene might be associated with obesity since it is upregulated in genetically obese rats. It is expressed in adipose tissue and strongly regulated by nutrition. When mice and rats are fed a high carbohydrate diet the mRNA expression is increased dramatically in white adipose tissue while fasting results in almost undetectable expression levels. In humans a similar pattern has been seen in patients on very low calorie diet. The aim of the study was to investigate the importance of seven chosen SNPs located in the ADPN gene in human obesity.
Materials and Methods: An obesity case-control material consisting of 234 pairs matched for age and gender (lean [age 43 (34–53) years, BMI 22.6 (21.3–23.8) kg/m^2] obese [age 42 (33–52) years, BMI 40.3 (35.5–45.3) kg/m^2]) were genotyped for seven SNPs in the ADPN gene using TaqMan assays on the ABI 7900HT (Applied Biosystems).
Results: There were no differences in either allelic or genotypic frequencies between lean and obese subjects for C-1375T located in the 5´UTR region, for Gly115Cys located in exon 2, for C+6237A located in intron 3 or for C+22707T located in the 3´UTR. Ile148Met in exon 3 differed significantly in allelic frequency, but there was no difference in genotypic frequency. There was a significant difference in both allelic and genotypic frequency for both C+9459T located in intron 5 and for A+16691G in intron 8. Haplotype analysis resulted in two blocks with very high LD (Ile148Met/C+6237A and C+9459T/A+16691G/C+22707T). The effect on obesity was reduced when analysing the blocks, indicating that individual SNPs had greater effect on obesity than the haplotypes.
Conclusion: Variation in the ADPN gene is associated with obesity.
Genotype frequencies of SNPs in the adiponutrine gene

SNP	Lean (%)			Obese (%)			P-value*
	11	12	22	11	12	22	
C-1375T	95.7	4.3	0.0	95.7	4.3	0.0	1.00
Gly115Cys	78.6	20.9	0.4	77.4	21.8	0.9	0.82
Ile148Met	52.1	42.3	5.6	61.1	35.5	3.4	0.12
C+6237A	36.1	52.4	11.6	36.5	46.4	17.2	0.18
C+9459T	61.4	35.6	3.0	72.1	25.3	2.6	0.046
A+16691G	56.8	39.7	3.4	70.9	26.1	3.0	0.0056
C+22707T	59.8	36.3	3.8	57.3	35.0	7.7	0.20

* Calculated using chi-square test. Values <0.05 is considered significant.

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Latent autoimmune diabetes in adults

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GAD -positivity in relatives of type 2 diabetes or LADA – results from the Botnia Study

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Background and Aims: GAD antibodies (GADA) identify and separate an insulin-deficient subgroup from the common form of type 2 diabetes (T2D). This subgroup is also called LADA. It is though not known whether GADA predict subsequent diabetes also in a nondiabetic population. Our aim was thus to study the value of GADA in non-diabetic (ND) relatives of patients with T2D to predict diabetes.
Materials and Methods: We analysed GADA with a radioimmunoprecipitation method in 5594 non-diabetic subjects from the Botnia Study, including 4713 relatives with (ND_{REL}) and 881 spouses without (ND_{CONT}) family history of diabetes.
Results: GADA were found in 4.9% of ND_{REL} and 5.1% of ND_{CONT}. The median GADA level of 46 IU/ml was significantly (P<0.00001) lower than that seen in T2D (80.5 IU/ml, N=174) or T1D (228 IU/ml, N= 272). The presence of GADA was not associated with any clinical characteristics at baseline. Among the 2300 ND subjects followed for 6 yrs, GADA *per se* (N=152) was not associated with deterioration of glucose tolerance when analysed with the Cox regression model. However, those repeatedly GADA+ during the follow-up (N=51) had a higher risk of developing early-onset (<45 yrs) diabetes (HR 5.6, 95% CI 1.2–25.6, P=0.026) compared to GADA- and/or GADA+ at single occasion. When the GADA positive subjects were stratified according to the GADA concentration, those with highest levels (upper quartile) developed diabetes significantly more often than those with from the three lower quartiles or without GADA: 21% (6/29) vs. 7.3% (9/123) vs. 5.6% (121/2148), P=0.0023. Also, among the GADA+ subjects, those with family history of T1D developed diabetes more often than those without such family history irrespective of GADA level [18% (8/45 vs. 8% (17/226), P=0.0361].
Conclusion: Low-level positivity of GADA is not associated with a clinical phenotype. However, non-diabetic subjects with high GADA levels or persistent GADA positivity have an increased risk of developing early-onset diabetes A novel finding was also that GADA positive individuals with a family history of T1D had an increased risk of T2D.

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Susceptibility for latent autoimmune diabetes in adults (LADA) is determined by variation at the *IDDM2* (insulin-gene) locus in white Caucasian patients from UK repositories

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Background and Aims: Latent autoimmune diabetes in adults (LADA) is a slowly-progressing form of autoimmune diabetes developing in adults that masquerades as clinical Type 2 diabetes (T2D). LADA is characterised by the presence of islet cell autoantibodies that occur in classical juvenile-onset Type 1 diabetes (T1D), although insulin dependence is not inevitable. Common disease pathology but a less aggressive clinical progression between LADA and classical T1D could arise from increased protection/reduced susceptibility from shared risk genes. Our aim was to determine whether the fine structure of the known association between islet autoimmunity and insulin-gene variation in T1D is also observed in LADA. In T1D, VNTR alleles detected by certain haplotypes in the insulin gene region were originally reported to confer differential susceptibility to disease. The following haplotypes were defined by the -23*HphI*, +1428*FokI* and +3580*MspI* sites: class I ID- and class I other (IC+/ID+), class III IIIA/'Protective' (PH) and IIIB/'Very Protective' (VPH) haplotypes. We tested for association of these T1D-defined haplotypes with LADA.
Materials and Methods: 442 antibody-positive, LADA patients aged >25 years at diagnosis (238 from UKPDS, 138 from Warren 2 consortium, 42 from Exeter Young-onset T2D study and 24 from Diabetes in Families study) and 353 non-diabetic subjects (ND) recruited in the Diabetes in Families study were genotyped for the -23*HphI* (class I/III VNTR detection), +1404*Fnu4HI* (+1428*FokI* surrogate, PH/VPH detection) and +3580*MspI* (ID- haplotype detection) variants by Amplifluor™ technology. Genotypic and haplotypic association tests were performed using standard contingency tables and haplotype trend regression, respectively. Haplotype frequencies were estimated using the estimation-maximisation (EM) algorithm, as implemented in the HelixTree™ software package.
Results: Single-point analyses revealed dominant protective effects at all three sites; T allele (class III) at -23*HphI* (OR = 0.43 [0.32-0.58], $p < 0.001$), A allele at +1404*Fnu4HI* (OR = 0.51 [0.37–0.69], $p < 0.001$) and C allele at +3580*MspI* (OR = 0.55 [0.37–0.83], p = 0.004). Variable levels of linkage disequilibrium (LD) (r^2 0.06–0.89) were observed between pairs of SNPs in the different sample groups. Four major 3-point haplotypes were observed with frequencies higher than 1%, corresponding to the class I ID-, class I other (IC+/ID+), PH and VPH. Haplotype frequency distributions differed significantly between LADA and ND (haplotype trend regression, $p = 5 \times 10^{-6}$). The haplotype frequencies in LADA vs ND were as follows: class I ID-, 40% vs 33%; class I other, 41% vs 37%; PH: 14% vs 22% and VPH: 4% vs 8%.
Conclusion: Class I haplotypes predispose to disease in LADA, whereas class III haplotypes confer a protective effect suggesting that susceptibility at the insulin gene region is similar to that reported for T1D.

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Relationship of autoantibodies to glutamic acid decarboxylase (GADA) to deterioration of glycamic control assessed by therapy progression in latent autoimmune diabetes in adults (LADA) in the UKPDS

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Background and Aims: Autoantibodies to glutamic acid decarboylase 65 (GADA) are markers of islet autoimmunity present at diagnosis in patients with type 1 diabetes (T1D) and latent autoimmune diabetes in adults (LADA). GADA persist after diagnosis in T1D for longer periods than other islet autoantibodies. However, the role of GADA in disease progression as a marker for β-cell failure in autoimmune diabetes is unclear. The aim of this study was to determine the persistence of GADA post-diagnosis and the relationship of GADA titre to disease progression in LADA.
Materials and Methods: GADA titres were determined in plasma samples from 242 subjects initially diagnosed with Type 2 diabetes in the United Kingdom Prospective Diabetes Study (UKPDS), (aged 25–65 yrs, ketonuria <3 mmol/l, no immediate insulin requirement) who were later found to be antibody-positive (ICA/GADA) and were defined as LADA patients. Subsequently, the same therapy decisions were made for antibody-positive patients as antibody-negative patients, defined by UKPDS protocol (diet → oral agents → insulin). GADA titres (WHO units) were measured at 0.5, 3 and 6 years post-diagnosis (p.d) by radiobinding assay. Deterioration of glycaemic control (>15 mmol/l) was assessed by progression within the therapy protocol; for this analysis, patients were termed as 'progressors' or 'non-progressors' at each of the three time-points.
Results: GADA positivity persisted until at least 6 yrs post-diagnosis. Median titres (IQR) of GADA were as follows: 0.5 yrs p.d, 331 (134–674); 3 yrs p.d, 199 (96–318); and 6 yrs p.d, 284 (107–518).
The proportion of patients by each timepoint on diet : oral agent : insulin therapy was follows: 0.5 yrs, 20% : 41% : 39%; 3 yrs, 6% : 29% : 65%; 6 yrs, 4% : 19% : 74%. GADA titre was not found to be significantly different between the patients in the therapy groups at any time-point.
By the 0.5 yr time-point, 23% of patients were 'progressors' and had higher GADA titres than 'non-progressors' (p<0.05); at diagnosis, 'progressors' were younger (p<0.025) and had higher fasting plasma glucose values (p=0.001).
Progression within the protocol by 3 and 6 yrs p.d. was not significantly related to GADA titre at either time-point; the median (IQR) titre of 'progressors' vs 'non-progressors' (between 0.5 - 3 yrs) was 184 (91, 316) vs 192 (81, 318), and between 3 - 6 yrs, 312 (93, 847) vs 250 (104, 464).
Conclusion: GADA postivity was maintained in LADA throughout the 6 yr follow-up period studied. Elevated GADA titres are predictive of require-

ment for more intensive therapy early in the course of disease but not at later time-points.
This study was kindly funded by Diabetes UK

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Vitamin D supplementation in adults with latent autoimmune diabetes (LADA)
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Background and Aims: Epidemiological data from the EURODIAB study showed that vitamin D (Vit D) supplementation has protective effects on T1DM. IMDIAB XI Trial suggested that Vit D treatment at diagnosis in children with type 1 diabetes can be beneficial in improving residual beta cell function. The aim of this study was to evaluate the effect of VitD3 intervention on pancreatic β cell function in adults with latent autoimmune diabetes (LADA).
Materials and Methods: LADA patients, defined as GAD-Ab positive phenotypic T2DM, with a fasting C peptide of 0.2 nmol/L or more and with duration less than 5 years, were enrolled and assigned to receive oral hypoglycemic agents (OHA) or insulin (INS). The patients in OHA group were treated with OHA alone (OHA group, n=18) or OHA combined withα-D3 0.25ug Bid (OHA+VitD group, n=15), and those in INS group were given subcutaneous insulin alone (INS group, n=12) or insulin combined with α-D3 0.25ug Bid (INS+VitD group, n=17). Blood was drawn every 6 months to determine plasma glucose, HbA1c and C peptide at fasting (FCP) and 2 hours after taking 75g glucose (PCP) without medication to compare the changes of islet βcell function. Serum 25(OH)D3 concentration was detected with high performance liquid chromatogram-mass spectrography (LC-MS). GAD-Ab and C peptide were measured with radioimmune assays.
Results: All of the 62 LADA patients were followed up for 6 months, 56 for 12 months and 27 for 18 months. (1) In the 4 groups of treated patients, HbA1c dropped significantly after 6 months of therapy. (2) In OHA group, FCP (0.71 vs 0.52 nmol/L,P=0.055)and HOMA-IS (129.0 vs 62.7, P=0.009) increased during the 6 months'observation. And in OHA+VitD group, FCP (0.67 vs 0.52 nmol/L,P=0.03) increased after 12 months' of therapy. At other time points of observation, there were no significant changes of FCP, PCP, ΔCP, HOMA-IS and HOMA-IR in OHA or OHA+Vit D groups. Between OHA+VitD group and OHA group, there were significant differences in ΔFCP (0.18 vs –0.07, P=0.039; 0.19 vs –0.12, P=0.011; 0.21 vs -0.02, P=0.016) after 6,12,18 months of therapy. (3) In INS group, FCP (0.29 vs 0.47, P=0.018) decreased after18 months of therapy. PCP (0.61 vs 1.33, P=0.028) decreased at 12 months as compared with baseline. While in INS+VitD group, FCP, PCP and ΔCP were steady during the 18 months. There were significant differences in ΔFCP (0.01 vs –0.80, P=0.009; 0.06 vs –0.21, P=0.011; 0.04 vs –0.27, P=0.022) at 6,12,18 months between INS+VitD group and INS group. (4) Although serum 25(OH)D3 and calcium concentration increased, and phosphorus levels decreased significantly at 6,12 months, they were all within normal ranges in vitamin D treated groups. No adverse effects were noted.
Conclusion: The preliminary data indicates that vitamin D supplementation can preserve pancreatic beta cell function in LADA patients as combined with OHA or insulin.
Support: National Natural Science Foundation of China

OP 40
Islet transplantation

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Donor islet endothelial cells participate in formation of functional vessels within pancreatic islet grafts
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Background and Aims: Pancreatic islet transplantation has emerged as a therapy for type 1 diabetes and is today performed using both freshly isolated and cultured islets. The islet blood vessels are disrupted during islet isolation, therefore proper revascularization of the transplanted islets is of great importance for the islet graft function and survival. Our aim was to study intraislet endothelial cells (ECs) after islet isolation, during islet culture and following islet transplantation.
Materials and Methods: Islets were isolated from transgenic Tie2-GFP mice, characterized by an EC specific expression of GFP, and living ECs could be studied in intact islets following isolation utilizing two-photon laser-scanning microscopy (TPLSM). By applying a novel *ex vivo* model for simultaneous perfusion and TPLSM imaging of the graft-bearing kidney, the morphology and the buffer flow through the islet graft vasculature could be imaged.
Results: Intraislet ECs were found to survive islet isolation but to rapidly disappear during islet culture. Following transplantation of freshly isolated Tie2-GFP islets, GFP fluorescent ECs were found to extensively contribute to vessels within the islet graft vasculature. Real-time imaging of the flow through the islet graft vasculature confirmed that the donor-derived vessels were functionally integrated.
Conclusion: Donor intraislet ECs have the capability of participating in the revascularization of transplanted pancreatic islets. Therefore, preservation of intraislet EC mass may improve long-term graft function.
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Blocking IFN-γ signaling cascade in islets alters their chemokine expression, but is insufficient for protection *in vivo*
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Background and Aims: The β-cell is an active participant in its own destruction in the pathogenesis of type 1 diabetes as well as after islet transplantation. Understanding the way β-cells die may provide clues for intervention aimed at making stronger β-cells. Exposure of β-cells to inflammatory cytokines such as IL-1β and IFN-γ leads to β-cell death through the activation of gene networks under control of specific transcription factors, such as IL-1β-dependent NFκB and IFN-γ-dependent STAT-1. We have demonstrated that β-cells lacking STAT-1 are resistant to cytokine-induced cell death *in vitro*. *In vivo*, however, other immune mechanisms besides cytokines are involved in β-cell destruction, suggesting a more aggressive process. In the present study, we investigated the effect of an isolated interruption of the IFNγ-signalling cascade by elimination of STAT-1 on β-cell behaviour and survival in models of immune-mediated cell destruction *in vivo* (allogeneic islet rejection and autoimmune disease recurrence).
Materials and Methods: Freshly isolated islets (n = 500) from STAT-1-/- and wild type (C57BL/6) mice were transplanted under the kidney capsule of alloxan-diabetic BALB/c or spontaneously diabetic NOD mice. Graft function was followed by daily blood glucose measurements. In a separate experiment, mice were killed 8 or 48 hours after transplantation and grafts were isolated for quantitative RT-PCR.
Results: Elimination of STAT-1 resulted in lower intra-graft expression levels of IL-15, IP-10 and iNOS 8 and 48 hours after grafting in both alloxan-diabetic BALB/c and spontaneously diabetic NOD mice. No difference was seen in IL-1β, IFN-γ, MCP-1 and MIP-3α. This was exactly the same pattern found when islets were exposed to cytokines *in vitro*. Although *in vitro* a total protection against cytokine-induced β-cell death was observed, no protection was seen *in vivo*. When transplanted in alloxan-diabetic BALB/c mice, STAT-1-/- islets (n = 6) were destroyed as rapidly as wild type islets (n = 13) (MST 12.4 ± 1.0 days *vs* 12.7 ± 2.7 days, p = NS). Also when islets were transplanted in spontaneously diabetic NOD mice no difference in survival was seen between STAT-1-/-

islets (n = 4) and wild type islets (n = 10) (MST 9.8 ± 2.9 days *vs* 9.7 ± 6.0 days, p = NS).
Conclusion: Although blocking of the IFN-γ signaling cascade by eliminating STAT-1 clearly alters β-cell behaviour and fully protects them against cytokine-induced cell death *in vitro*, this single intervention is not sufficient for protection of β-cells in allograft rejection and autoimmune disease recurrence after islet transplantation. This approach resulting in altered β-cell vulnerability *in vitro* may however contribute to better *in vivo* results when combined with some immunosuppression.
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Over-expression of activated AMP-activated protein kinase (AMPK) impairs *in vivo* pancreatic β-cell function in transplanted pancreatic islets

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Background and Aims: Islet cell transplantation is now a realistic treatment modality for Type 1 diabetes but is limited by donor tissue shortage. Reduction of immediate apoptotic losses and increasing glucose responsiveness of the transplanted beta cell mass would therefore be advantageous. Since increases in AMP-activated protein kinase (AMPK) activity in β-cells suppress insulin secretion and prompt apoptosis of β-cells *in vitro*, we hypothesised that activated AMPK may be detrimental for graft function whereas enzyme inhibition may be beneficial. We demonstrate that adenovirus-mediated expression of activated AMPK (a) suppresses glucose metabolism and glucose-stimulated insulin secretion in isolated pancreatic islets and (b) suppresses the recovery from hyperglycaemia achieved by islet transplantation into diabetic mice.
Materials and Methods: Primary rat (male Wistar, 250g) and mouse (C57 BL/6; 8–14 wks, 20–25g) islets were isolated after *in situ* collagenase digestion, and cultured for 16 h (transplantation) or 48 h (*in vitro*) with one of three adenoviruses: AdeGFP (null virus), AdAMPK CA (constitutively active AMPKα1/2 [1–312] $T^{172}D$) or AdAMPK DN (dominant negative AMPKα1; $D^{157}A$) at a multiplicity of infection of 50–100. Efficiency of infection was assessed by confocal microscopy for GFP fluorescence and was typically 20–30%. AMPK activity (phosphopeptide transfer), [U-^{14}C] glucose oxidation, glucose-stimulated insulin secretion (static incubation for 30 min. and radioimmunoassay) and caspas-3 immunodetection were assayed using standard protocols. Syngeneic C57 BL/6 mice rendered diabetic with streptozotocin (160 mg/kg) were transplanted under the kidney capsule using a suboptimal transfected islet mass (300 islets/animal), and graft function monitored by blood glucose analysis at intervals of one to two days and intraperitoneal glucose tolerance tests (2g/kg). Statistical analysis was performed by unpaired student's t-test or one-way ANOVA with post-hoc testing for glycaemic control.
Results: Assayed in isolated islets, insulin secretion stimulated by 17 (*versus* 3) mmol/l glucose was inhibited by 36.5% (three independent experiments; $p < 0.01$) and 43% ($p < 0.02$) by over-expression of AMPK CA *versus* null (GFP alone) in mouse and rat islets, respectively. A similar decrease in glucose oxidation was also observed in each case (38%, $p < 0.05$ and 26.6%, $p < 0.05$, respectively). Neither parameter was significantly affected by introduction of AMPK DN. However, western (immuno-) blot analysis showed a reduction in caspase-3 cleaved products in murine islets expressing AMPK-DN compared to null or AMPK CA islets. In eight cohorts of transplanted animals (24 transplants), impairment in glycaemic control was seen in those animals transplanted with islets expressing AMPK CA in comparison to both null ($p < 0.01$) and AMPK DN infected grafts ($p < 0.01$). Glucose tolerance tests performed at day 30 post transplantation on contemporaneous cohorts showed a more rapid return to euglycaemia in animals with islets expressing AMPK DN than AMPK CA ($p = 0.009$) or null ($p < 0.04$) viruses.
Conclusion: We conclude that activation of AMPK has detrimental effects on both beta cell function and apoptotic index *in vitro* and *in vivo*. Suppression of AMPK activity may therefore provide a useful means to enhance graft function after human islet transplantation.
Support: Wellcome Trust

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Homeostatic expansion of autoreactive T-cells in patients undergoing immunosuppression after islet transplantation

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Background and aims: Islet transplantation is a potential cure for patients with type I diabetes but recurrent autoimmunity upon antigen re-exposure may compromise graft survival. We hypothesized that under immunosuppressive regimen patients undergo T-cell loss and compensatory homeostatic proliferation that could selectively expand autoreactive T-cells.
Materials and methods: Thirteen patients who underwent solitary islet transplantation with anti-IL-2R monoclonal antibody induction therapy followed by FK506 and rapamycin maintenance therapy (Edmonton protocol) were studied.
Results: Patients showed reductions of $CD3^+$T-cells immediately post-transplant and increased serum concentrations of homeostatic cytokines such as IL-7 and IL-15. Ki-67 staining, a marker of ongoing proliferation, demonstrated between 1 and 3% of peripheral CD4+ and CD8+ cells actively proliferating *in vivo* after transplant compared to <0.4% prior to transplant ($P < 0.0001$). Ki-67 positive cells were memory CD45RO pos cells. Staining with an MHC-classI-GAD65 tetramer demonstrated that GAD autoreactive T-cells were 10–100-fold enriched in the Ki-67+ population. Although the combination of FK506 and rapamycin failed to prevent in vivo expansion of autoreactive T-cells, rapamycin was effective in inhibiting IFN-γ production, and removal of rapamycin and FK506 resulted in Ki-67+ cells rapidly becoming IFN-γ+. Switching patients to micophenolate-mofetil (MMF) therapy reduced Ki-67 positivity to pre-transplant levels, and MMF was able to block IL-7 induced proliferation in vitro.
Conclusions: The data suggest that homeostatic cytokines released under immunosuppression can induce proliferation of total T-cells and favor the expansion of autoreactive memory T-cells in patients with type 1 diabetes who undergo islet transplantation. This expansion, which appears to be controlled with the cytostatic drug MMF, may contribute to the long term loss of graft function in these patients.

OP 41

Regulation of metabolism: role of the brain

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Fatty acid uptake in various brain regions measured with [^{11}C]-palmitate, [^{18}F]FTHA and PET

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Background and Aims: Brain free fatty acid (FFA) uptake and metabolism are currently insufficiently defined. Animal and human data have demonstrated that injected [^{11}C]-palmitate is rapidly taken up by the brain tissue but there is debate on the fate of the tracer in brain cells as well as the mechanism through which it is taken up. [^{11}C]-palmitate can be used as a marker of FFA oxidation and uptake, and [^{18}F]-fluoro-6-thiaheptadecanoic acid ([^{18}F]FTHA) has been proposed as a marker of the β-oxidation pathway.
Materials and Methods: Eight healthy male subjects were studied in fasting state. [^{11}C]-palmitate or [^{18}F]FTHA was given intravenously as a 1-minute bolus injection and dynamic brain PET imaging for 30–60 minutes was started simultaneously. Magnetic resonance imaging was used as reference to localize brain regions. ROIs (regions of interest) were drawn to cerebellum, cortex, hypothalamus, pituitary, pons, thalamus, and striatum using Vinci 1.82. program which allows fitting of the PET and MRI images. Venous plasma time-activity curve was used as input function and Gjedde-Patlak plot was used for quantification of Ki. Plasma concentration of FFAs was determined during the PET scanning and was multiplied with Ki to determine FFA uptake in various brain regions.
Results: With [^{11}C]-palmitate FFA uptake was in average 2.6-times higher in pituitary (3.9 ± 0.6 micromol/100g per min) than in other brain regions (thalamus: 1.5 ± 0.2 micromol/100g per min) in all subjects ($p = 0.0002$ between pituitary and thalamus, paired T-test). Uptake (micromol/100g per min) in thalamus, striatum (1.5 ± 0.2), cerebellum (1.4 ± 0.2), cortex (1.5 ± 0.2), hypothalamus (1.4 ± 0.2) and pons (1.4 ± 0.2) did not differ from each others. Oxidation of palmitate occurred in all brain regions. With [^{18}F]FTHA, fractional uptake (Ki) of the tracer was higher in cortex (0.0145 ± 0.0020) and cerebellum (0.0158 ± 0.0014) than in pituitary (0.0084 ± 0.0019; $p < 0.01$ and $p < 0.05$, respectively).
Conclusion: [^{11}C]-palmitate seems to be feasible for the measurement of fatty acid uptake in human brain regions. [^{11}C]-palmitate uptake and oxidation in pituitary may be of importance in signaling between peripheral tissues and higher brain regions.

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Regional differences in brain glucose metabolic response to basal insulin: the effects of insulin resistance

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Background and Aims: Insulin resistance syndrome includes glucose intolerance, type 2 diabetes and obesity. Dysregulation of cerebral networks involved in appetite control and behavioural motivation, including ventral striatum, amygdala and hypothalamus, has been implicated in the pathogenesis of obesity. We have recently described an insulin sensitive component to human brain glucose metabolism and demonstrated that this is diminished in systemic insulin resistance. We investigated whether regional differences in human brain insulin responsiveness involve cerebral appetite and motivational networks, and whether these are affected by insulin resistance.
Materials and Methods: Quantitative brain glucose metabolism was measured using ^{18}F-fluoro-deoxyglucose positron emission tomography (FDG-PET) in 14 non-diabetic men on two occasions in random order. The subjects were categorised for insulin sensitivity based on HOMA-IR. Endogenous insulin was suppressed by somatostatin infusion in both studies and in one, basal insulin was replaced by insulin infusion ($0.3\,mU \cdot kg^{-1} \cdot min^{-1}$). Regional differences in the action of insulin on brain glucose uptake were analysed by statistical parametric mapping after correction for global metabolic rate.
Results: Somatostatin suppressed plasma insulin (2 ± 2 mU/l) and insulin infusion restored post-absorptive concentrations (24 ± 4 mU/l). The effect of replacing basal insulin on cerebral metabolic rate for glucose (CMRglu) was significantly greater in ventral striatum, particularly on the right (Talairach coordinates x y z: 22 10 -4; k=329; Z=4.62) and in regions of prefrontal cortex and anterior cingulate ($p<0.001$, corrected), relative to the rest of the brain. In contrast, right amygdala (20 -2 -28, k=550, Z=4.21) and cerebellar vermis showed decreased response to insulin ($p<0.001$, corrected). The increase in global cerebral glucose metabolic rate with insulin was inversely correlated with insulin resistance as measured by fasting insulin concentrations ($r = -0.569$, $p = 0.034$).
Conclusion: Our data demonstrate that basal insulin signalling is associated with acivation of bilateral ventral striatal regions and deactivation of the amygdala, areas involved in appetite and motivation. As insulin resistance inversely correlated with cerebral metabolic rate, our data are compatible with the hypothesis that insulin resistance alters the set point for triggering appetite and food related motivational behaviour. We conclude that there are functional abnormalities of activation of brain regions important in appetite control and feeding behaviour in human subjects with insulin resistance.
Support: Diabetes Research and Wellness Foundation and Diabetes UK

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Cerebral GLP-1 regulated insulin secretion, liver and muscle glycogen synthesis

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Background and Aims: GLP-1 is released by the intestine into the hepatoportal blood in response to glucose and lipids absorption where it controls the portal glucose sensor. However, GLP-1 is also produced in the brain but its role on the cerebral glucose sensor has not been identified.
Materials and Methods: We performed hyperglycemic 5, 10 or 20 mM glucose clamps for 3 hours in mice in the presence or absence of a cerebral coinfusion of the GLP-1 receptor agonist Exendin 4-39 (Ex4).
Results: In these conditions, plasma insulinemia and liver glycogen deposition were increased 5 and 2 fold respectively. However, when in addition to hyperglycemia, hyperinsulinemia was maintained similar between groups, by the mean of an exogenous insulin infusion, muscle glucose utilization was reduced in the Ex4 infused mice when compared to saline.
Conversely, when the GLP-1 receptor antagonist Exendin 9-39 (Ex9) was infused into the brain muscle glucose utilization and glycogen synthesis were further increased in hyperglycemic conditions only while liver glycogen was unchanged. Furthermore, muscle glycogen synthase kinase 3β was inhibited by phosphorylation leading to the activation of the glycogen synthase. These effects were independent from hyperinsulinemia since the same data were obtained in mice deleted from the insulin receptor specifically in the muscles (MIRKO). Importantly, the increased muscle glucose transport effect of Ex9 could bypass insulin resistance since a four week continuous delivery of intraperitoneal Ex9 via osmotic minipumps improves glucose tolerance of high fat fed insulin resistant mice.
Conclusion: Our data suggest that during systemic hyperglycemia the cerebral GLP-1 receptor favors hepatic glycogen deposition by increasing insulin secretion and preventing muscle glucose utilization and glycogen synthesis.

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Central effects of fatty acids on glucose-induced insulin secretion and hypothalamus gene expression is related to their chain length and degree of saturation

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Background and Aims: Fatty acids (FA) play a key role in regulation of glucose metabolism through their effect on central nervous system activity. Indeed, FA modulate food intake as well as hepatic glucose production and glucose-induced insulin secretion (GIIS). We previously showed that intracarotid infusion of triglycerides lead to increased GIIS and that beta-oxidation in the brain was required for the effects of FA (Diabetologia, 2004, 47(11):2032). However, this central effect of FA in relation with their chain length or degree of saturation is poorly understood. We studied the effect of 48 h central infusion of oleate, octanoate or linolenate on food

intake and insulin secretion in response to glucose. In order to identify some target within hypothalamus, we also measured gene expression of key proteins involved in FA metabolism.
Materials and Methods: Rats received an intracerebroventricular infusion of FA. Briefly, rats were stereotactically implanted with a chronic stainless steel cannula in the right lateral cerebral ventricle. The cannula was connected via a polyethylene catheter to a subcutaneously osmotic minipump filled up with FA (oleate, octanoate or linolenate) or saline. Infusions started 6 h after surgery. The rate of infusion was 0.5 µl/h. Blood was daily removed (~ 80 µl) from caudal vessels for measurement of plasma substrate (FA and glucose) and insulin. Food intake was daily measured. At day 3 of infusion GIIS was measured in response to a single intraperitoneal injection of glucose. In another serie of experiments, etomoxir (CPT1 inhibitor) was concomitantly infused with FA. At the end of experiment brain were removed and five hypothalamus nuclei (arcuate, lateral, ventromedian, paraventricular and dorsomedian) were micropunched in order to measure gene expression (acetylCoA carboxylase, ACC, carnitine palmitoyl transferase, CPT1, FA synthase, FAS, G protein related peptide GPR41).
Results: Whatever the FA, there was no change in food intake during experiment. Plasma glucose, FA, and insulin concentrations were also similar in all groups. In response to glucose load, octanoate-infused rats showed a glucose intolerant state which was not compensated by an increased GIIS. In linoleate group time course of glycemia was similar to control but was associated with an increased GIIS, suggesting an adaptation of pancreatic B-cell to insulin-resistance. This effect of linolenate on GIIS was reversed by etomoxir. Finally in oleate group there was no change in GIIS. Hypothalamic gene expression were also modified in FA infused rats. For example, FAS expression was inhibited in all nuclei except in VMH of linolenate infused rats. GPR41 was up-regulated in ARC of linolenate infused rats.
Conclusion: In conclusion, central FA effects on insulin secretion and action are related to their chain length or degree of saturation. Such effects are also mediated through differential changes in gene expression.

OP 42

Advanced glycation end products: basic mechanisms and complications

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The receptor RAGE regulates glyoxalase-1 transcription, expression and activity

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Background and Aims: Formation of Advanced Glycation End Products (AGEs), binding of AGEs to the receptor RAGE and subsequent RAGE dependent cellular activation are supposed to contribute to the development and progression of late diabetic complications. Analogous, AGE-detoxifying cellular mechanisms such as glyoxalase-1 (GO-1) activity might lower RAGE-dependent cellular dysfunction by preventing AGE-formation mediated by methylglyoxal, glyoxal and other alpha-oxoaldehydes.
Materials and Methods: In order to study a suspected interaction between RAGE and GO-1, GO-1 transcription (Real time PCR), -expression (Western Blot) and -activity (Enzyme assay) was determined in different organs from healthy and diabetic wildtype and RAGE-deficient ($RAGE^{-/-}$)-mice and in cultured fibroblasts isolated from these mice strains
Results: Transcription, expression and activity of GO-1 was significantly higher in kidneys, nerves and skin of $RAGE^{-/-}$-mice, when compared to RAGE-bearing wildtype (WT) mice. Diabetes reduced GO-1 transcription in both, WT- and $RAGE^{-/-}$-mice, but the decrease was less prominent in $RAGE^{-/-}$-mice. Cell culture experiments using isolated fibroblasts confirmed higher basal GO-1 transcription in the absence of RAGE. Incubation with AGE-albumin (but also other RAGE ligands such as S100B) reduced GO-1 in WT cells, while inducing GO-1 in $RAGE^{-/-}$-cells in a time and dose dependent manner. Consistently, we observed a significant increase in cellular methylglyoxal-derived AGEs in wildtype-fibroblasts stimulated with AGEs and/or methylglyoxal, but not in $RAGE^{-/-}$ derived fibroblasts.
Conclusion: These data demonstrate for the first time, that engagement of RAGE suppresses AGE mediated induction of GO -1 transcription, expression and activity.

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Renal intra-mitochondrial glycation drives deficiencies in the activity of manganese superoxide dismutase and complex I of the mitochondrial respiratory chain in diabetes

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Background and Aims: Advanced glycation end products (AGEs) are postulated to be involved in the pathogenesis of diabetic nephropathy. In the diabetic kidney, mitochondria are direct targets for increased glucose concentrations. High intracellular glucose concentrations accelerate AGE formation. The aim of this study was to determine if intra-mitochondrial glycation contributes to mitochondrial dysfunction.
Materials and Methods: Rats were rendered diabetic by streptozotocin (50 mg/kg) and followed for 16 or 32 weeks. Mitochondria were isolated from the renal cortex and various parameters of mitochondrial function were assessed.
Results: In diabetes, circulating carboxymethyllysine (CML, an important AGE in vivo) concentrations were increased at both 16 and 32 weeks ($p<0.01$). In the renal mitochondria of the animals with diabetes, CML content was two-fold greater compared to sham (control) rats ($p<0.01$), indicating clearly the presence of intra-mitochondrial glycation. Complex I of the mitochondrial respiratory chain in isolated glomeruli was diminished ($p<0.05$), along with manganese-containing superoxide dismutase (MnSOD) activity ($p<0.05$) at both 16 and 32 weeks. In late diabetes, both superoxide radical production ($p<0.01$) and nitrotyrosine concentration ($p<0.05$) were also elevated. Renal glutathione peroxidase activity ($p<0.02$)

was increased early but suppressed late in diabetes, whilst hydrogen peroxide production remained unchanged.
Conclusion: In conclusion, this study demonstrates that AGEs formed within mitochondria during diabetes contribute to mitochondrial dysfunction via suppression of MnSOD and Complex I of the respiratory chain. This increases production of the superoxide radical, shunting the process towards the peroxynitrite pathway. Activation of this cascade may be partly responsible for AGE-related kidney dysfunction in diabetic nephropathy.
Support: Juvenile Diabetes Research Foundation

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Do extracellular (circulating or dietary) advanced glycation end products mediate mitochondrial dysfunction in the kidney?
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Background and aims: There is an increasing body of evidence to support the concept that advanced glycation end products (AGEs) are involved in the pathogenesis of diabetic nephropathy. In addition, circulating levels of AGEs, from metabolism, the diet or extracellular formation have been shown to correlate with declining renal function. The aim of this study was to determine if these extracellular AGEs are taken up by the kidney for processing/excretion and contribute to mitochondrial dysfunction.
Materials and methods: Male Sprague Dawley rats were given AGE-modified rat serum albumin intraperitoneally (20 mg/kg/day; 38 mmol/mol lysine carboxymethyllysine) for 16 weeks. Control rats were given unmodified rat serum albumin (20 mg/kg/day; 0.3 mmol/mol lysine carboxymethyllysine). A subset of rats was also treated with ALT-711 (10 mg/kg/day), an AGE cross-link breaker or idebenone, a mitochondrial free radical scavenger (100 mg/kg/day). Mitochondria were isolated from the kidney and various parameters of mitochondrial function were assessed. In addition, normal rat kidney tubule cells (NRK52E) with or without AGE treatment were incubated with Mitotracker red (a fluorescent oxidant-sensitive dye), and membrane potential was observed by confocal microscopy.
Results: In the animals with AGE infusion, cytosolic carboxymethyllysine (CML, an important AGE in vivo) content was increased ($p<0.001$), whilst mitochondrial CML was decreased ($p<0.01$). Both ALT-711 and idebenone restored these parameters to normal ($p<0.001$). Complex I of the mitochondrial respiratory chain and MnSOD activity were both suppressed by AGE infusion ($p<0.001$). Both ALT-711 and idebenone normalised MnSOD activity, although did not affect Complex I activity. Mitochondrial membrane potential was markedly altered by AGE treatment in NRK52E cells, indicating the ability of AGEs to affect mitochondrial metabolism.
Conclusions: In conclusion, this study demonstrates that AGEs, when exogenously administered independent of hyperglycaemia can induce mitochondrial dysfunction via decreased activity of Complex I and MnSOD. These AGE-mediated events may represent an additional mechanism for kidney dysfunction in diabetic nephropathy. The specific mechanism for uptake of AGEs into the cells remains to be determined.
Support: Juvenile Diabetes Research Foundation

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RAGE -374 T/A polymorphism is associated with type 1 diabetes and late diabetic complications
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Background and aims: The receptor for advanced glycation end-products (RAGE) is mainly considered as an intracellular signal-transducter or pro-inflammatory peptide of possible importance for inflammation and autoimmune diseases. No previous study has compared frequency of RAGE polymorphisms in type 1 and type 2 diabetes but associations between functional RAGE polymorphisms and diabetic nephropathy and retinopathy have been described. Our aim was to study a putative association with RAGE -374 T/A polymorphism, both in relation to type of diabetes and diabetic complications.
Materials and methods: The RAGE -374 T/A polymorphism was genotyped using allelic discrimination method in 860 Type 1 and 2453 type 2 Scandinavian diabetic patients from the local diabetes registry. HLA typing was carried out using time-resolved fluorimetry and the frequency of the RAGE polymorphism was studied in different HLA-DQB1 genotypes and correlated to diabetic nephropathy, retinopathy, neuropathy and macrovascular complications.
Results: Type 1 diabetic patients had a lower frequency of the wild type homozygous T/T genotype than type 2 diabetic patients (48.9% vs. 55.1%, $p=0.002$). The HLA-DQB1 genotype was associated with the RAGE -374 T/A polymorphism. Type 1 diabetic patients with risk genotypes (02/0302, 0302/X, 0302/0604) had significantly lower frequency of T/T genotype than patients with neutral or protective HLA-DQB1 genotypes (38.5% vs. 63.2%, $p<0.000001$).
In type 1 diabetes the frequency of the wild type genotype was lower in patients with diabetic nephropathy than in those with normal albumin excretion rate (38.6% vs. 53.3%, $p=0.006$). Type 1 diabetic patients with sight-threatening retinopathy also had a lower frequency of the wild type genotype T/T than patients without sight-threatening retinopathy (43.8% vs. 52.1%, $p=0.03$). In type 2 diabetes, the association with RAGE polymorphism seemed to be dependent of the metabolic control and in patients with low HbA_{1c}, the wild type genotype seemed to be more frequent in patients with complications, than in patients without complications: diabetic nephropathy (61.5% vs. 44.9%, $p=0.02$) and diabetes macrovascular complications (60.7% vs. 50.9%, $p=0.04$).
Conclusions: RAGE -374 T/A polymorphism is associated with type 1 diabetes and the HLA-DQB1 genotype. The RAGE -374 T/A polymorphism is also associated with diabetic complications suggesting that this association is dependent on both the metabolic control before the onset of complications and type of diabetes.

OP 43

Insulin therapy in type 1 diabetes

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Regular insulin is as effective as rapid-acting insulin analogs in combination with glargine insulin in type 1 diabetic patients

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Background and Aims: Glargine (G) insulin due to its peak-less profile and less risk of hypoglycemia had displaced NPH insulin as first-line basal insulin in type 1 diabetes. Although in multiple daily injection (MDI) regimens for type 1 diabetes rapid-acting insulin analogs are used commonly in combination with G, no evidence exist to recommend any particular insulin formulation as prandial insulin. The aim of the study was to compare human regular (Reg) vs lispro (Lp) vs aspart (Asp) insulin in combination with G in type 1 diabetic (T1D) patients.
Materials and Methods: 79 G-naïve T1D patients (age 32.3 ± 9.6 years-old, 46 women, BMI 24.8 ± 3.3 kg/m^2, diabetes duration 14.5 ± 7.9 y) under MDI therapy with NPH insulin were randomised in a 6-month prospective study to a treatment including Reg (n=26), Lp (n=25) or Asp (n=28) as prandial insulin and G at dinner or bedtime as basal insulin. Reg was administered 30 min before meals instead of Lp or Asp just after eating. All patients were similar instructed in carbs counting before starting in the study and adjusted insulin doses according to meal CH content. Although blood samples were obtained at 0, 3 and 6 months, only mean HbA_{1c} change, insulin doses, weight and hypoglycemic events after 6 months were shown. Results are presented as means±SD. Statistical evaluations were performed using parametric and non-parametric methods with a significance level of <0.05.
Results: Initial HbA_{1c} was comparable between the three groups (Reg-Lp-Asp): 8.6 ± 0.9 vs 8.3 ± 0.9 vs 8.5 ± 1.0%. After 6 months, mean HbA_{1c} was significantly reduced irrespective of the type of prandial insulin used (95 CI): Reg -0.63% (–0.23,-1.03), Lp -0.73% (–0.32,-1.15), Asp -0.49% (–0.10, -0.88), p=0.267 between groups. Total insulin doses (Reg-Lp-Asp, 46.3 ± 13.7 vs 46.9 ± 12.5 vs 52.4 ± 20.3 IU/day, p=0.31) and prandial insulin doses (22.6 ± 7.6 vs 20.0 ± 6.7 vs 24.2 ± 10.9 IU/day, p=0.32) remained constant during the study and were comparable between groups in the last visit. Daily G doses increased (~ 10%) significantly across the study and were higher with both rapid-acting insulin analogs at 6 months (23.5 ± 7.2 vs 27.8 ± 8.8 vs 27.9 ± 9.3 IU/day, ANOVA p=0.04, Reg-Lp p=0.051, Reg-Asp p=0.019). Weight did not change after 6 months with any of the treatments and total, mild and severe hypogycemic events were scarce and comparable between groups.
Conclusion: In T1D patients under MDI, substitution of NPH insulin for G as basal insulin led to significant reductions of HbA_{1c} irrespective of the type of prandial insulin chosen (Reg, Lp or Asp). However, when rapid-acting insulin analogs are used in combination with G insulin, higher basal insulin doses will be required in comparison with regimens using Reg as prandial insulin.

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Insulin detemir plus insulin aspart is associated with less risk of major as well as nocturnal hypoglycaemia than insulin glargine plus insulin aspart at comparable levels of glycaemic control in type 1 diabetes

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Background and Aims: The aim of this multi-centre, 1:1 randomised, open-label, parallel group trial was to compare glycaemic control, risk of hypoglycaemia and weight development after 26 weeks of treatment with insulin detemir (IDet) and insulin glargine (Glarg) as basal insulin in combination with pre-meal insulin aspart (IAsp).
Materials and Methods: Of 322 randomised men and women with type 1 diabetes, 320 were exposed to trial products (IDet: 161, Glarg: 159, sex: 51% males. Clinical characteristics (mean±SD) age: 40.2 ± 13.6 yrs, duration of diabetes: 16.7 ± 10.5 yrs, BMI: 25.5 ± 3.6 kg/m^2, HbA_{1c}: 8.8 ± 1.0%). Subjects administered IDet in the morning and at bedtime or Glarg at bedtime in combination with IAsp before each main meal. The first 6 weeks was regarded as a titration period and the remaining 20 weeks as a maintenance period. Pre-breakfast and pre-dinner plasma glucose targets were ≤7.3 mmol/L (131 mg/dL) and post-prandial target was ≤10.1 mmol/L (≤181 mg/dL) throughout the trial.
Results: HbA_{1c} decreased by 0.6% points in both groups and was comparable between treatments after 26 weeks at 8.2% in both groups, mean difference IDet-Glarg: –0.03% [95% CI: –0.246; 0.187]. The overall shape of the home-measured 9-point plasma glucose profile was similar between the two treatment groups (p=0.125). Home-measured fasting plasma glucose was lower with Glarg than with IDet: 7.01 vs 7.71 mmol/L after 26 weeks of treatment, p<0.001. Within-subject variation in home-measured pre-breakfast and pre-lunch plasma glucose was comparable between the two groups, (ns), while variation in pre-dinner plasma glucose was lower with IDet than with Glarg (p<0.05). The risk of major and nocturnal (23:00 to 06:00) hypoglycaemia was 72% and 32% lower with IDet than with Glarg (p<0.05) whereas the overall risk was similar between the groups. The overall safety profile was similar for the two treatments. Mean body weight increased by 0.5 kg with IDet and by 1.0 kg with Glarg after 26 weeks of treatment, (ns).
Conclusion: Treatment with insulin detemir compared to insulin glargine as basal insulin in combination with insulin aspart resulted in lower risk of major hypoglycaemic episodes as well as less nocturnal hypoglycaemia at a similar level of improvement in glycaemic control. This indicates that insulin detemir has therapeutic advantages when treating people with type 1 diabetes on a basal-bolus insulin regimen.
The study was sponsored by Novo Nordisk A/S

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Insulin detemir reduces the risk of hypoglycaemia at all levels of HbA_{1c} compared to NPH insulin in the context of basal-bolus therapy for type 1 diabetes

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Background and Aims: The incidence and progression of diabetic complications is known to be reduced when good glycaemic control is achieved. However, aggressive titration of insulin in pursuit of this goal increases the risk of hypoglycaemia and this risk ultimately limits the degree of control achievable. In comparative trials against NPH insulin, the basal insulin analogue insulin detemir has been consistently associated with reduced within-subject variability in pharmacodynamic effect, reduced weight gain and reduced risk of nocturnal hypoglycaemia. The present pooled analysis of multicentre, randomised, clinical trials involving people with type 1 diabetes (16–28 weeks treatment) was undertaken to assess whether insulin detemir favourably shifts the relationship between glycaemic control and overall hypoglycaemic risk at all levels of HbA1c in comparison to NPH insulin
Materials and Methods: The analysis included all poolable randomised studies in adult patients with type 1 diabetes where the marketed formulation of insulin detemir, used once or twice daily as the basal component of basal-bolus therapy, was compared to NPH insulin. A treatment-by-trial-interaction analysis was undertaken to ensure poolability of data and one trial out of five was excluded due to an atypically low hypoglycaemia rate. The mean number of total hypoglycaemic events (major: BG <2.8 mmol/L, assistance required, plus minor: BG <2.8 mmol/L, no assistance required) per patient occurring during the last 3 months of treatment was calculated and plotted as a function of HbA1c. Analysis of risk of hypoglycaemia by HbA1c and relative risk was modelled in a poisson model with gamma frailty and HbA1c as covariate.
Results: Poolable data were available for 1180 patients receiving insulin detemir and 810 receiving NPH insulin. Baseline demographic characteristics were similar by treatment. The overall relative risk for hypoglycaemia with insulin detemir was 0.78, representing a 22% risk reduction in comparison to NPH insulin (p<0.001). At all levels of HbA1c insulin detemir was associated with a lower risk for hypoglycaemia (table). When calculated hypoglycaemic events per patient year were plotted against HbA1c, the curves indicated that the extent of the risk reduction for hypoglycaemia with insulin detemir increased with improving glycaemic control. The single excluded trial did not contradict the findings of this pooled analysis.
Conclusion: These results suggest that treatment with insulin detemir should allow more patients to achieve good glycaemic control with a lower associated risk of hypoglycaemia in comparison to NPH insulin.

	HbA1c	7.0%	8.0%	9.0%	10.0%
Hypoglycaemic events per patient · year	NPH	47	34	25	18
Hypoglycaemic events per patient · year	Detemir	37	27	19	14

Support: Novo Nordisk

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Continuous subcutaneous insulin infusion reduces diabetes-related complications when compared with multiple daily injections, for type 1 diabetes treatment in Switzerland: a health economic analysis

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Objectives: The aim of this study was to compare the costs and clinical benefits of continuous subcutaneous insulin infusion (CSII) compared with multiple daily injections (MDI) for type 1 diabetes treatment (T1D) in Switzerland.
Methods: The effect of CSII and MDI treatment of type 1 diabetes on healthcare costs and clinical results in the Swiss setting was analyzed and the CORE Diabetes Model was used to predict costs and clinical outcomes. This is a peer-reviewed, validated model based on a series of sub-models that simulate the major complications of diabetes. Each sub-model is a Markov-based Monte Carlo simulation with transition probabilities derived from major clinical studies. Baseline cohort characteristics were taken from published sources. Clinical effectiveness of CSII and MDI was taken from a recent meta-analysis. Published Swiss costs for 2004, health care resource utilization and clinical data, and recommended discount rates were used (3.0% *per annum* on costs and outcomes). The analysis was run over a lifetime horizon from a societal perspective. Finally extensive sensitivity analyses were performed.
Results: Treatment with CSII was associated with an improvement in life expectancy (LE) of 0.87 years compared to MDI (mean LE 17.15 ± 0.20 versus 16.27 ± 0.20 years). This was accompanied by a decrease in cumulative incidence of severe vision loss, end stage renal disease (ESRD) and peripheral vascular disease (PVD), by 16%, 18% and 16% respectively (Table 1). Mean total lifetime costs were CHF 19'628 more expensive with CSII treatment versus MDI (CHF 516'745 versus CHF 497'117). This results in an incremental cost effectiveness ratio of CHF 22'444 per life year saved with CSII compared to MDI. The breakdown of costs showed that complications costs were CHF 10'327/patient lower in the CSII group. Sensitivity analysis on hypoglycemia rates showed that if we assume that CSII reduces hypoglycemic events the therapy is cost-saving in Switzerland (Table 2).
Conclusion: Improvements in glycemic control associated with CSII versus MDI lead to improvements in LE due to reduced incidence of diabetes-related complications. CSII is cost effective compared to MDI according to accepted international thresholds. **ICER= incremental cost-effectiveness ratio for CSII versus MDI, expressed in cost per quality-adjusted life year saved.*

Table 1: Decrease of complications after CSII therapy

Reduction of cumulative incidence of complications

Years after start of CSII therapy	Severe Vision Loss	First Amputation	ESRD	MI	PVD
10	21%	1%	30%	10%	36%
15	30%	9%	31%	17%	37%
20	32%	11%	38%	20%	36%
60	16%	0.4	18%	6%	16%

Support was received by Medtronic Europe S.A.

Table 2: Results of sensitivity analysis

Effect of CSII on hypoglycemia rate

Analysis	Life Expectancy (years)		QALE (years)		Total lifetime costs (CHF)		ICER*
	CSII	MDI	CSII	MDI	CSII	MDI	
Reduced by 62%	17.15 ± 0.21	16.27 ± 0.20	11.83 ± 0.15	10.92 ± 0.13	499'661 ± 6'814	497'117 ± 7'283	2'801
Reduced by 75%	17.14 ± 0.20	16.27 ± 0.20	11.83 ± 0.14	10.92 ± 0.13	496'682 ± 6'869	497'117 ± 7'283	–478

OP 44

Prediction and prevention of type 2 diabetes

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Genetic prediction of the metabolic syndrome in the Botnia study

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Background and Aims: The metabolic syndrome (MSDR) is a cluster of risk factors associated with cardiovascular morbidity and mortality with abdominal obesity and insulin resistance as common denominators. The syndrome results from a collision between genetic and environmental factors. Whereas the environmental triggers are well known, the genetic factors are not. The aim of the study was therefore to evaluate the role of common variants in candidate genes previously associated with insulin resistance or abdominal obesity for their ability to predict the metabolic syndrome in a large prospective study (the Botnia Study).
Materials and Methods: 2293 non-diabetic individuals, 1937 of whom had no MSDR at baseline (873 males/1064 females; age 44 ± 14 years; BMI 25 ± 4 kg/m^2) participating in the prospective study were genotyped for variants in the genes encoding for the *PPARG* (Pro12Ala), *Calpain-10* (*CAPN10* SNP-43, -44), muscle glycogen synthase (*GYS1* XbaI), β_1-(Gly389Arg), β_2-(Arg16Gly) and β_3- (Trp64Arg) adrenergic receptor and adiponectin (*APM1* SNP276,-2019). MSDR was defined using the NCEP ATP III criteria. Cox proportional hazard analysis was used to test whether the genotyped variants could predict development of MSDR.
Results: During a median 6-year follow-up, 267 (14%) individuals developed the metabolic syndrome. The *PPARG* Pro12Ala (Pro/Pro-genotype) (HR 1.5, 95% CI 1.1–2.0, P = 0.019) and β_1-*AR* Gly389Arg (Gly-allele) (HR 1.4, 95% CI 1.1–1.8, P = 0.006) variants were associated with increased risk of developing MSDR. Of the different MSDR components, the risk Pro/Pro-genotype of *PPARG* and Gly-allele of β_1-*AR* predicted elevated fasting P-glucose (≥ 6.1 mmol/l) (HR 1.3, 95% CI 1.0–1.8, P=0.031 and HR 1.3, 95% CI 1.0–1.6, P=0.045, respectively). A multivariate analysis of combined genetic effects revealed an additive effect of the *PPARG* Pro12Ala and β_1-*AR* Gly389Arg variants (P<0.001) on the risk of MSDR.
Conclusion: We demonstrate in a large prospective study that variants in the *PPARG* and β_1-*AR* genes in an additive way predict the metabolic syndrome.

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Does achievement of Diabetes Prevention Study (DPS) healthy goals protect against incident diabetes in a population-based study?

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Background and Aims: Intensive behavioural interventions aimed at high-risk people with impaired glucose tolerance have halved progression to diabetes in three different randomised trials. In the Finnish DPS study risk of diabetes was reduced by 58% through targeting lifestyle behaviours including nutritional intake, physical activity and weight reduction. It is unclear whether the same risk reduction might be realised if individuals in the general population could meet the same behavioural targets. We quantified the association between trial behaviour goals and diabetes incidence in a population-based cohort study.
Materials and Methods: EPIC-Norfolk is a prospective cohort of 24,155 participants aged 40–79 years who attended a baseline health check including anthropometric and blood pressure measurement, and completion of validated diet and physical activity questionnaires. Incident cases of diabetes were ascertained from multiple sources including follow-up health checks, hospital and general practice registers and prescribing data. Mean duration of follow-up was 4.6 ± 1.3 (SD) years. We assessed whether meeting five healthy 'goals' similar to those described in the DPS (BMI <25 kg/m^2, fat intake < 30% of energy intake, saturated-fat intake <10% of energy intake, fibre intake ≥15g/1000 kcal, physical activity >4 hrs/wk) was associated with reduced risk of developing diabetes.
Results: Only one-fifth of EPIC participants met 3 or more DPS goals. Those meeting a higher number of goals were more likely to be female, younger, less deprived and non-smokers than those meeting few goals (chi-squared test for trend p<0.001). Only 3.8% of participants reported consuming ≥15g/1000 kcal of fibre daily, but 76.0% reported engaging in physical activity for more than four hours a week. The incidence of diabetes was inversely related to the number of DPS goals achieved (p-value for linear trend <0.001). No participants who met all five goals developed diabetes, whereas diabetes incidence was highest in those who did not meet any goals (Table 1).
Conclusion: Population observational data support the findings of trials among high risk individuals -people who meet more of a set of five diabetes prevention health behaviour goals are less likely to develop the condition. Interventions that promote achievement of these goals, if successfully applied in the general population, could significantly reduce the growing burden of diabetes-related morbidity and mortality.

Table 1 Crude diabetes incidence rate stratified according to achievement of the five DPS goals

	DPS goals met					
	0	1	2	3	4	5
Number of cases	74	199	66	48	7	0
Rate/1000 p-years	6.4	4.5	1.9	3.5	1.1	–
95% CI for rates	5.1–8.0	3.9–5.2	1.5–2.4	2.7–4.7	0.5–2.3	–

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Clusters of components of the metabolic syndrome and adipose-derived mediators in the prediction of type 2 diabetes - no independent role of inflammation or dyslipidemia

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Aims: To generate a hypothesis on the structure of the metabolic syndrome (MetSy) and test the ability of composite factors to predict type 2 diabetes (T2DM).
Subjects and Methods: We performed a prospective study using baseline data from a population-based health survey (n=33,336) in the Umeå area in Northern Sweden. Baseline variables included plasma glucose (fasting and 2 h-OGTT), BMI, blood pressure, blood lipids (NEFA, total cholesterol, HDL-cholesterol, triglycerides), adipokines and inflammatory markers (leptin, IL-6, TNF-alpha, highly-sensitive CRP) and indices of insulin resistance and β-cell function (insulin, proinsulin, HOMA). 177 individuals that were non-diabetic at the health survey were later on diagnosed with T2DM (after a mean time of 5.4 years). Two referents, who did not develop diabetes during follow-up (mean observation time 8.1 years), were selected for each diabetes case. Exploratory and confirmative factor analysis was applied to describe the structure of the MetSy and the prediction of T2DM was evaluated by multivariate regression analysis.
Results: The hypothetical model generated by factor analysis comprised five intercorrelated composite factors. Among those, two factors significantly and independently predicted T2DM; OR for an obesity/insulin resistance factor was 5.1 and 3.4 in males and females, respectively, and for a glycemia factor 4.7 and 5.3, respectively. In contrast, the inflammation, dyslipidemia and blood pressure factors were predicitive only in univariate but not in multivariate analysis. Interestingly, none of the five composite factors improved the prediction of T2DM as compared to single variables (BMI, proinsulin and FPG).
Conclusion: In the complex pathophysiology of the metabolic syndrome, obesity with accompanying insulin resistance in combination with β-cell decompensation are the core perturbations promoting progression to T2DM. In contrast, inflammation, dyslipidemia and blood pressure are not independent risk markers for the development of T2DM.
Support: Swedish Research Council

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Progression from impaired glucose tolerance to type 2 diabetes in the Finnish Diabetes Prevention Study: predictive power of immune markers

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Background and Aims: In recent years, evidence accumulated that a low-grade inflammation plays an important role in the pathogenesis of type 2 diabetes (T2D). The Finnish Diabetes Prevention Study (DPS) showed that multiple modest lifestyle changes including weight loss, dietary changes and increased physical activity reduce the incidence of T2D by 58% in individuals with impaired glucose tolerance (IGT). In this study, we test the hypothesis that systemic concentrations of immune mediators at DPS baseline predict susceptibility or resistance to type 2 diabetes prevention by lifestyle intervention.
Materials and Methods: The study participants were randomly assigned to the intervention group (n=257) or the control group (n=265). Individuals in the control group were given general oral and written information about diet and exercise at the start of the study and at subsequent annual visits, whereas subjects in the non-pharmacological intervention group received intensive individualised counseling to achieve weight loss, change in dietary behaviour and increase in physical activity. This study is based on a follow-up period of 3.9 years. At baseline, the immunological parameters CRP, SAA, IL-6, RANTES, MIF and sICAM-1 were measured.
Results: CRP was the best immune marker for the prediction of incident T2D in the control group only. In the intervention group, T2D incidence despite lifestyle changes was significantly higher in subjects with the highest RANTES concentrations and was significantly lower in subjects with the highest MIF levels. Individual ratios of RANTES/MIF in the upper tertile were highly predictive of T2D incidence in the intervention group (p=0.003) and less pronounced in the control group (p=0.088). Moreover, study participants with high RANTES/MIF values exhibited in general a less favourable one-year development of various metabolic parameters compared to subjects with lower RANTES/MIF ratios.
Conclusion: In summary, serum concentrations of immune markers were found to predict susceptibility or resistance to T2D prevention by nonpharmacological intervention based on diet and exercise in the DPS. Our data indicate that immunological phenotyping might reveal additional information about patients with increased risk of developing T2D as assessed by 'traditional' metabolic risk factors.

OP 45
Lipid metabolism

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Molecular screening of the lipoprotein lipase gene in hypertriglyceridemic members of type 2 diabetic pedigrees

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Background and Aims: Lipoprotein lipase(LPL) is responsible for the hydrolysis of triglyceride(TG) -rich lipoproteins, and LPL mutations may result in the hypertriglyceridemia. Hypertriglyceridemia is common among individuals with type 2 diabetes mellitus(T2DM).To test the hypothesis that the LPL gene mutations are responsible for the hypertriglyceridemia in some individuals with T2DM, we investigate the LPL gene mutations in hypertriglyceridemic members of type 2 diabetic pedigrees in Chinese population.
Materials and Methods: Members of type 2 diabetic pedigrees were chosen for analysis of LPL gene if fasting TG levels exceeded 2.25 mmol/l. Fifty-three individuals met the criteria, including 31 individuals with T2DM, 11 individuals with impaired glucose tolerance (IGT), and 11 individuals with normal glucose tolerance(NGT). These subjects represented 26 type 2 diabetic pedigrees. TG levels ranged from 2.3 mmol/l to 13 mmol/l. The control population consisted of 118 individuals chosen from non-diabetic families. Polymerase chain reaction(PCR)-single strand conformation polymorphism(SSCP), PCR-denaturing high performance liquid chromatography(DHPLC) and direct DNA sequencing were applied for analysis of LPL gene.
Results: **1.** A total of 7 different LPL variants were detected among 53 individuals of type 2 diabetic pedigrees. Four of the variants were previously detected in exon 3 (Ala71Thr and Val108Val), and exon 6 (Leu286Pro and Asn291Ser). Three novel variants were detected in exon6 (Lys312insertion C) and in exon 8 (Thr361insertion A and Leu376Leu). **2.** The LPL gene in the family members of the novel Lys312insC and Thr361insA mutations pedigrees were further analyzed. In 8# family, 6 individuals who carried Lys312insC mutation had a high triglyceridemia,and three of them also carried the Asn291Ser mutation had a more high triglyceridemia,so maybe there was a strong linkage disequilibrium between the Lys312insC mutation and the Asn291Ser mutation; In 14# and 28# family,the Thr361insA mutation cosegregated with the phenotype (hypertriglyceridemia) and was thus suggested to have a pathophysiological effect**;** No individual carried the Lys312insC and Thr361insA mutations in control group. **3.** In new 1# pedigree, the proband whose TG was 11.9 mmol/l was found carrying the Leu286Pro mutation; and in new 3# pedigree, the proband whose TG was 13 mmol/l was found carrying the Ala71Thr mutation. The mutation, Leu286Pro and Ala71Thr, reported previously, had a significant association with hypertriglyceridemia, so we think these mutations contribute to the hypertriglyceridemia in this two pedigrees.
Conclusion: The genetic variants at the LPL gene occur commonly in hypertriglyceridemic members of type 2 diabetic pedigrees in Chinese population. LPL variants contribute to the hypertriglyceridemia in some type 2 diabetic families.

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T111I polymorphism of endothelial lipase and HDL-C in the D.E.S.I.R. cohort

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Background and Aims: Endothelial lipase (EL) is a new member of the lipase gene family. EL has been shown to play a significant role in modulating the concentrations of plasma high density lipoprotein (HDL) levels but its function is not clearly understood. Overexpression of EL decreases HDL cholesterol levels whereas blocking its action increases concentrations of HDL cholesterol. Genotypic variants in the EL gene have been identified. The most common SNP of the EL gene, C584T, produces a protein variant: threonine at 111 changes to isoleucine (T111I). The aim of this study was to investigate the influence of T111I polymorphism on lipid levels in a large general population. Overweight and high insulin levels are associated with

low HDL-C concentrations. Therefore, influence of the T111I polymorphism according to these factors has also been tested.
Materials and Methods: The study population consisted of 2,576 men and 2,636 women aged 30–65 years who volunteered in Health Examination centres in the western central part of France: D.E.S.I.R. (Data from an Epidemiological Study on the Insulin Resistance syndrome). Clinical, anthropometric and biologic data were collected at baseline. The SNP was genotyped using PCR amplification followed by hybridization with fluorescent specific allelic probes (molecular beacons). Association of genotypes with quantitative variables was tested by ANOVA or ANCOVA adjusted for age, gender, body mass index (BMI), insulin. Two groups have been defined according to BMI: (normal weight with BMI <25 kg/m^2 / overweight: BMI ≥25 kg/m^2).Three groups according to insulin tertiles were defined: tertile I = insulin ≤4.42 µU/mL; tertile II = insulin >4.42 µU/mL and <6.96 µU/mL; tertile III = insulin ≥ 6.96 µU/mL.
Results: The genotype distributions were in Hardy-Weinberg equilibrium with allele frequencies similar to those reported in other Caucasian populations (rare allele I frequency = 0.32). The T111I polymorphism was associated with HDL-C concentrations in the whole cohort. Subjects carrying I allele had higher HDL-C than the TT carriers (TT genotype: 1.61 ± 0.42 mmol/L, TI: 1.64 ± 0.43 mmol/L, II: 1.68 ± 0.44 mmol/L; P = 0.001). The same association was observed with apolipoprotein AI (apo AI), the HDL major protein (TT: 1.63 ± 0.26 g/L; TI: 1.65 ± 0.26 g/L, II: 1.67 ± 0.27 g/L; P = 0.001). Stratified analysis by BMI revealed that this influence was statistically significant in normal weight subjects (n = 2,959): P = 0.02 and P = 0.001 for HDL-C and apo AI, respectively. There was no association in overweight subjects. Stratified analysis by insulin tertile showed that this association persisted only in subjects with low insulin (<6.96 µU/mL, n = 3,383): P = 0.003 and P< 0.001 for HDL-C and apo AI, respectively.
Conclusion: This study suggests that in the general population, T111I polymorphism of the endothelial lipase modulates the HDL-C and apo AI concentrations. This association disappears in subjects with overweight and high insulin levels.

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Abnormal postprandial chylomicron response and decreased adipose tissue lipoprotein lipase activity in type 2 diabetes are independent of insulin resistance

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Background and Aims: Postprandial lipoprotein abnormalities in type 2 diabetes are associated with insulin resistance. The role of other diabetes-related factors is still not clear. The aim of this study was to evaluate possible additional specific effects of type 2 diabetes on postprandial dyslipidemia and the role of adipose tissue, particularly of lipoprotein lipase (LPL).
Materials and Methods: Ten obese diabetic, 11 obese non diabetic, and 9 normal weight control male subjects, aged between 30 and 60 years, with normal fasting plasma triglyceride levels, were administered a fat-rich standard meal (944 kcal, 57% fat, 31% CHO, 12% proteins). Fasting and postprandial blood samples were taken for lipoprotein analysis after separation by discontinuous density gradient ultracentrifugation. A needle biopsy was taken to obtain abdominal subcutaneous adipose tissue 6 h after meal as well as, on a different day, in the fasting condition before a hyperinsulinemic euglycemic clamp (1.5 mU insulin · kg b.w. · min) was performed.
Results: Obese subjects with diabetes and obese without diabetes had similar levels of insulin resistance (M/I ratio 2.5 ± 0.4 and 1.5 ± 0.2; M±SEM) compared with controls (8.8 ± 1.4). The two obese groups showed a similarly higher postprandial increase of large VLDL lipids (triglyceride incremental area under the curve -IAUC- 153 ± 18 and 123 ± 20 mg/dl · 6 h) than controls (81 ± 15 mg/dl·6 h, p<0.05). Diabetic subjects, compared with non diabetic obese subjects, also had an increased postprandial chylomicron response (triglyceride IAUC 113 ± 21 vs. 64 ± 11 mg/dl · 6 h, p<0.05), as well as a reduced adipose tissue heparin released LPL activity, both at fasting (105 ± 14 vs.196 ± 42 nmol FA/g adipose tissue/h, p<0.05) and postprandially (112 ± 18 vs. 202 ± 43 nmol FA/g adipose tissue/h, p<0.05). Adipose tissue LPL mRNA was, instead, not significantly different between obese subjects with and without diabetes, both in the fasting and postprandial condition.
Conclusion: In the insulin resistant conditions of obesity with and without diabetes large VLDL lipids are increased after a fat-rich meal. In addition, diabetic patients, compared with non diabetic obese subjects, have an increased chylomicron response associated with a reduction of adipose tissue LPL activity.

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Postprandial (pp) metabolic markers during a standardized test meal following 12 weeks on premeal insulin lispro (LP) plus bedtime NPH or twice-daily NPH in patients with type 2 diabetes

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Background and Aims: To determine whether control of pp hyperglycemia with a prandial insulin regimen also improves pp lipid profiles.
Materials and Methods: Two insulin regimens were used to explore acute pp changes in metabolic markers associated with cardiovascular (CV) disease. An open-label, randomized, 2-period (12 weeks), crossover study was used to compare a prandial regimen (premeal insulin LP + bedtime NPH) with a basal regimen (NPH twice daily). Thirty patients with type 2 diabetes (12 F, 18 M, mean age 61 yrs, mean diabetes duration 16 yrs) were randomized after a 2-month lead-in with NPH bid (post lead-in HbA1c: 8.4 ± 0.9%).
Results: After 12 weeks, the LP regimen resulted in lower HbA1c (7.6 vs 8.2%, p<0.001) compared with NPH twice daily, without increasing hypoglycemia (incidence 22 vs 30%, p=NS) or insulin dose (0.45 vs 0.53 U/kg, p=0.052). A lunch test meal (mean 1242 ± 460 kcal: 50% carbohydrate, 40% fat, 10% protein) was individualized based upon caloric needs and held constant across periods. LP was administered before the test meal while NPH was given prior to breakfast. Following the test meal, LP was associated with lower pp glucose ($AUC_{0-5\ hr}$ 43.5 vs 57.6 mM-hr, p<0.001), and an early decrease followed by a rise in free fatty acids (1 hr: 0.27 vs 0.36 mM, p=0.002; 5 hr: 0.48 vs 0.39 mM, p=0.046). No significant differences were found in triglycerides (4 hr: 2.45 vs 2.71 mM, p=0.087) or total cholesterol (5 hr: 5.38 vs 5.20, p=0.077), but low-density lipoprotein cholesterol was lower (5 hr: 3.12 vs 2.88 mM, p=0.012), and 5-hr high-density lipoprotein cholesterol was significantly higher than NPH (1.15 vs 1.07 mM, p=0.004).
Conclusion: Improved pp glycemic control as obtained with LP in a prandial insulin regimen is associated with significantly lower HbA1c and acute modulation of lipid fractions following a test meal that may have a favorable impact on CV risk in patients with type 2 diabetes.

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Screening for peripheral neuropathy

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The development of peripheral neuropathy over two years – results from a community-based cohort

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Background and Aims: The detection of early signs of peripheral neuropathy is essential if foot complications in diabetes are to be avoided. This study investigates differences in the incidence of neuropathy that may be obtained using the clinical tools commonly used in primary care screening during the patients' annual review.
Materials and Methods: A random sample of 1097/9710 (11.3%) participants recruited to the North West Diabetes Foot Care Study received neuropathy screening at baseline and were followed up two years later. At both time points, tests for neuropathy consisted of the modified Neuropathy Disability Score (NDS), [a composite of vibration perception at the hallux (128 Hz tuning fork), Sharp/blunt pain perception at the hallux (Neurotip), Temperature perception using hot and cold rods and ankle reflex response] and 10g monofilament insensitivity.
Results: At baseline, 316/1097 (28.8%) were found to have a normal response in all modalities but after two years 33% of these 316 patients presented with some evidence of peripheral neuropathy. 10/103 (9.71%) developed insensitivity to 10g monofilament, pain perception was reduced in 14/103 (13.6%), temperature perception was reduced in 18/103 (17.5%), 26/103 (25.2%) lost vibration perception and an ankle reflex response was completely absent in 44/103 (42.3%) at the end of the two year period. When compared with any deficit recorded by the NDS over two years, the sensitivity of monofilament was 0.34: 95% CI (0.30,0.39); 0.60: 95% CI (0.55,0.64) for vibration perception alone and 0.63: 95% CI (0.59,0.68) for a loss of monofilament and/or vibration perception. The highest observed sensitivity value was for the ankle reflex response 0.94: 95% CI (0.92,0.96).
Conclusion: The diagnosis of peripheral neuropathy varies considerably depending on the method used. These results suggest that a sizeable proportion of patients at risk of foot problems are likely to be missed, if the screening relies on the 10 g monofilament and/or the 128Hz tuning fork. Multi-modality testing, therefore, is recommended, particularly when screening for early signs of peripheral neuropathy in diabetes.

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International Working Group's Diabetic Foot Risk Classification: validation in a large population based cohort

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Background and Aims: The aim of the study was to evaluate the effectiveness of the diabetic foot risk classification system proposed by the International Working Group on the Diabetic Foot (IWGDF) to predict foot ulcers, amputations and hospitalizations.
Materials and Methods: We evaluated 1,666 consecutive diabetic patients enrolled in a managed-care-based outpatient clinic in San Antonio, Texas, for an average of 27.2 ± 4.2 months (range 20–32) for a total of 43,024 member months. At enrollment, patients underwent a standardized general medical examination and detailed foot assessment, and were educated about proper foot care; they were then re-screened at scheduled intervals, and also seen promptly if they developed any foot problem. Patients and their families were provided diabetic specific education, therapeutic shoes and insoles and regular foot care. We used the IWGDF diabetic foot risk classification to stratify ulcers.

Results: Incidence of complications over 27 month evaluation period. Numbers are in percentages.

	No Disease	Neuropathy	Neuropathy and/or Deformity	Peripheral Vascular Disease	History of Ulceration	History of Amputation
Ulceration	4.3	12.6	7.4	30.3	70.3	74.2
Hospitalization	0.1	1.9	0.4	14.5	9.9	45.5
Amputation	0	1.5	0	8.5	5.5	42.4
– at Foot Level	0	1.0	0	3.0	4.4	21.2
– at Leg Level	0	0.5	0	5.5	1.1	21.2

There was a significant trend for more ulcerations, amputations and hospitalizations as risk group increased (x^2 for trend $p<0.001$). In addition, when patients with a history of ulceration or amputation were stratified by peripheral vascular disease (PVD), there were significantly more ulcers and amputations in patients with PVD ($p<0.01$).
Conclusion: The IWGDF Diabetic Foot Risk Classification can help predict future complications: However, additional stratification for the presence of PVD within groups and history of amputation can further help to identify subjects at increased risk.

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Asian diabetic patients have fewer foot deformities compared to age- and sex-matched European diabetic patients

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Background and Aims: Previously, we found that Asians with diabetes in the UK have a 3-fold lower rate of foot ulceration than their European counterparts. We now aimed to examine foot deformity differences between these groups.
Materials and Methods: Data from 110 Asian and 110 European randomly selected age- and sex-matched diabetic patients, who were part of a larger community-based foot screening programme, were analysed. During foot screening, patients' feet were examined for small muscle wasting, hammer/claw toes, bony prominences, prominent metatarsal heads, Charcot arthropathy and limited joint mobility during prayer sign (LJM). Each deformity scored 1 when present or 0 when absent, on either foot.
Results: Mean foot deformity score was lower in Asians (1.1 ± 1.1 [SD]) than Europeans (2.1 ± 1.6, $p<0.0001$). Prevalence rates of deformities in these groups, respectively, were: small muscle wasting – 25% vs. 29%; hammer/claw toes - 17% vs. 46% ($p<0.0001$); bony prominences – 33% vs. 43%; prominent metatarsal heads – 11% vs. 42% ($p<0.0001$); Charcot – 0% vs. 0.9%; LJM – 26% vs. 44% ($p=0.007$).
Conclusion: European diabetic patients have 4-fold greater prevalence of prominent metatarsal heads than Asians and 3-fold greater prevalence of hammer/claw toes. Joint mobility is nearly twice as often impaired in Europeans. These factors probably contribute to the much higher rate of ulceration in European patients and highlight the importance of abnormal areas of higher pressure in ulcer aetiology.

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A comparison of screening approaches for diabetic peripheral neuropathy

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Background and Aims: Screening for diabetic microvascular complications (DMC) is a standard of care for diabetes (DM) patients. There is, however, little agreement as to the best approach to screening for diabetic peripheral neuropathy (DPN), a common DMC. We undertook the current study to determine the sensitivity of available tests alone or in combination in screening for early DPN.
Materials and Methods: We performed systematic DPN screening tests in 206 consecutive subjects completing an annual diabetes care assessment (mean±SD age was 57 ± 13 years, DM duration 9 ± 9 years, 84% type 2 DM, A1C 7.3 ± 1.4%). Tests included nerve conduction studies (performed on non-dominant peroneal nerve by automated device), Michigan Neuropathy Screening Instrument (MNSI), 4 and 10 g monofilament, quantitative sen-

sory testing of vibration detection threshold (VDT), vibration testing with tuning fork (measuring the delay in vibration perception between patient's foot and clinician's hand), and both a patient and health care provider-administered Neuropathy Total Symptom Score-6 (NTSS-6). DPN was defined as MNSI score >2.0 plus either VDT≥95th percentile or abnormal nerve conduction studies (defined as either <5th percentile Distal Motor Latency and F-wave Latency, or >95th percentile for compound muscle action potential). Other non-DPN data included random urine albumin/creatinine ratio (≥30 mg/g = nephropathy) and non-mydriatic retinal photo of each eye (evaluated for retinopathy by a retinal specialist by AAO guidelines).
Results: DMCs (including DPN, retinopathy and nephropathy) were identified in 48% of the 166 patients with complete data (39.2% with one DMC, 8.4% with two DMCs). Nearly one-third (31%) of subjects with complete neuropathy data met the definition of DPN. Abnormal nerve conduction studies were found in 66% of patients, and this was used as the reference for evaluating sensitivity of other DPN screening tests. The 10 g monofilament was least sensitive (16% sensitivity), compared to all other tests including the 4 g monofilament (31%). The most sensitive screening tests were MNSI>0 (82% sensitivity), patient-administered NTSS6>0 (74%), and tuning fork exam (63%). DPN screening was more sensitive when instruments were used in combination, as shown below.

Pair of Tests		Sensitivity*
	HCP-administered NTSS-6 >2	83/114=73%
	HCP-administered NTSS-6 >0	97/114=85%
	Patient-administered NTSS-6 >0	100/113=88%
Tuning Fork plus:	MNSI >2	75/115=65%
	MNSI >0	97/115=84%
	4 g monofilament	79/113=70%
	10 g monofilament	75/113=66%

* Likelihood that at least one test out of the pair of tests will detect DPN

Conclusion: DMCs were detected in half of the patients in our survey despite short duration of DM and relatively good glycemic control. DPN was the most common DMC. The standard 10 g monofilament, a test often conducted in clinical practice, while effective in detecting advanced DPN, identified only half of those with DPN identified by other means and was the least sensitive of all instruments evaluated. These observations support more consistent screening for early DPN using a systematic approach that includes an early clinical test for neuropathy such as the tuning fork exam and an evaluation of symptoms of neuropathy in patients with and without known DMCs.
Study funded by Eli Lilly and Company

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Markers of autoimmunity

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Identification of insulin B chain epitopes linked to type 1 diabetes susceptible HLA-DQ2 and -DQ8 CIS and trans dimers
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Background and Aims: To identify any effect of HLA-DQ2 and -DQ8 cis- and trans-dimers on T-cell recognition of insulin B chain epitopes associated with HLA-DQ susceptibility alleles.
Materials and Methods: T cells from a DQ8 homozygous newly diagnosed Type 1 diabetes patient were cultured and cloned in vitro in the presence of the well-characterized insulin B9-23 peptide, presented by antigen-presenting cells expressing HLA-DQ, previously shown to be diabetogenic in NOD mice. Using the coordinates of the crystal structures of HLA-DQ8 (DQA1*0301/B1*0302) and -DQ2 (DQA1*0501/B1*0201) we modelled the fitting of various registers of this peptide in the groove of the HLA-DQ2, and the trans-dimers DQA1*0501/B1*0302 and DQA1*0301/B1*0201.
Results: Type 1 diabetic patient-derived T cells recognized and proliferated to this insulin peptide in the context of the diabetes-susceptible HLA-DQ alleles HLA-DQA1*0301/B1*0302 (DQ8). Intriguingly, the additional presence of DQA1*0501/B1*0201 (DQ2) or DQA1*0102/B1*0602 (DQ6.2) affected the proliferative response and cytokine production profile. Homology modeling indicated that the best fit of the peptide in the groove of DQA1*0501/B1*0201 is for the core nonamer B8-16, while the HLA-DQA1*0501/B1*0302, and A1*0301/B1*0201 trans-dimers allow the best fit to the B13-21 nonamer. In all three cases the MHC II-peptide surface that will be in contact with the cognate T cell receptor differs with respect to surface residues and electric charge, making unlikely the possibility of T cell recognition of two or more of these combinations by the same clone.
Conclusion: T cells of newly-diagnosed type 1 diabetes patients potentially recognize insulin B chain peptides in the context of the three susceptibility alleles HLA-DQA1*0501/B1*0201, A1*0501/B1*0302 and A1*0301/B1*0201. This makes the insulin B chain as that most laden with antigenic sequences sensitized to T cells in combination with the HLA-DQ susceptibility alleles. It is remarkable that the dominantly protective allele DQA1*0102/B1*0602 binds to the sequence Insulin B6-14, overlapping in part to all sequences that bind to various HLA-DQ susceptibility alleles. These results reveal a particular degree of immunogenicity of insulin B chain with respect to diabetes-susceptible HLA-DQ alleles, and are consistent with the hypothesis of epitope stealing as the mechanism of dominant protection from the disease.
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Detection of GAD_{65}-specific T cells by class I tetramers in newly diagnosed type 1 diabetes (T1D) patients
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Background and Aims: T1D is an autoimmune disease, in which the insulin-producing pancreatic β cells are destroyed in genetically predisposed individuals. The pre-clinical onset is characterized by the presence of circulating autoantibodies that are directed against islet-specific autoantigens. While the direct contribution of autoantibodies to the disease pathogenesis is controversial, it is generally accepted that the mechanism of β cell destruction is mediated by autoreactive T cells that had escaped the thymic selection. Attempts have been tried but failed in standardizing a reliable assay for measuring circulating islet autoreactive T cells. In order to explain these difficulties, several explanations have been put forward, including the low frequency of circulating autoreactive T cells, mainly because recruited within the pancreas at the disease onset.

Alternative explanations are the characteristics of autoantigenic proteins employed in the various assays. The aim of this study was therefore to design a reliable method to detect circulating CD8+ /GAD autoreactive T cells ex vivo in newly diagnosed T1D patients; the HLA class I tetramers was the technology implemented.
Materials and Methods: PBMC of 22 HLA-A2 T1D patients and of 21 HLA-A2 matched normal controls were cultured in the presence of a high affinity HLA-A2 binder peptide from GAD_{65} or IL-2. Both GAD-specific stimulated cells and IL-2 treated cells were stained with PE labeled tetrameric complexes and then analysed by flow cytometry at day 6. A high affinity HLA-A2 binder peptide from Flu was used as antigen control.
Results: Low frequencies of circulating CD3+/CD8+/GAD_{65} reactive T lymphocytes were detected in PBMC of normal controls both in basal condition (IL-2 stimulation) (in average 0.3% of CD3+/CD8+ cells) and after GAD peptide-specific stimulation (in average 0.54% of CD3+/CD8+ cells). Conversely, high frequencies of CD3+/CD8+/GAD reactive T cells were detected in PBMC of 19 out of 22 T1D patients after GAD peptide stimulation (in average 30.5% of CD3+/CD8+ cells); frequencies of cytotoxic T cells were higher than in basal condition (IL-2 stimulation) (in average 4.6% of CD3+/CD8+ cells). One out of the 3 GAD-unresponsive diabetic PBMC (6 day assay) was exposed to a shorter course of GAD stimulation (2 days); in this experimental condition, a high frequency of CD3+/CD8+/GAD reactive T cells (12.48% versus 1.17% after IL-2 treatment) was detected. The total population of CD3+/CD8+/GAD reactive T cells of T1D patients after GAD peptide stimulation showed two subpopulations with different CD8 intensities; this pattern was not present in the population of controls after peptide stimulation. CD8+/GAD reactive cells expressed the early activation marker CD69 and a percentage of this population was also Annexin V positive. These phenomenona can be interpreted as a consequence of T cell activation.
Conclusion: The evidence presented indicates that we have developed a reliable assay for detecting GAD_{65} autoreactive T cells in PBMC of T1D patients. This finding now offers the possibility to introduce the test in the prediabetic period in order to monitor the events leading to β cell destruction.

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Study of T cell response to Ii-Key/MHC class II epitope hybrid peptides in human type 1 diabetes
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Background and Aims: Type 1 diabetes (T1DM) is a chronic, T cell-mediated autoimmune disease that results in the destruction of the insulin-producing beta cells of the pancreas. HLA class I and class II molecules play a major role in the presentation of short, pathogen-derived peptides to T cells, a process that initiates the adaptive cellular and humoral immune responses.
A novel approach to study antigen presentation by HLA Class II is based on immunoregulation by Ii protein of peptide charging to HLA class II molecules. It has been shown that a peptide of the Ii protein (Ii-Key) enhances *in vitro* presentation of antigenic peptides, thus creating a therapeutic opportunity to regulate the antigenic peptide binding to MHC Class II molecules. The aim of our study was to apply this technique to testing the use of Ii-Key/MHC class II insulin epitope hybrids in characterizing anti-insulin T cell responses in patients with T1DM.
Materials and Methods: Peptides of insulin shown by others to be recognized by CD4+ T cells of T1DM patients were selected from the literature. Predicted DRB1*0301 and DRB1*0401 epitopes were identified with the Rammensee SYFPEITH program (http://syfpeithi.bmi-heidelberg.com/Scripts/ MHCServer.dll/EpPredict.htm) applied to the insulin peptides. Within a homologous series of hybrids with nested deletions from the N-termini of the epitope-containing segment, the longest and shortest hybrids were assayed with the shortest epitope-only peptides as controls. Five series (15 hybrids and five epitope-only controls) were analyzed. Peripheral blood mononuclear cells (PBMC) were assayed from 17 patients (9 male) mean age of 35 years (age range 15–58 years) affected by T1DM, with mean disease duration of 20 years (range 10–26 years), and 8 normal subjects (5 male) with mean age of 39 years (age range 15–57 years). PBMCs were cultured with PHA as positive controls and all the Ii-Key hybrids as well as epitope-only controls for both IFN gamma analysis by BD ELISPOT and T cell proliferation measured by tritiated thymidine incorporation.
Results: Both basal IFN gamma and proliferation were normal in patients with T1DM, and there was no significant difference between patients and controls. After PHA stimulation, both IFN gamma production as well as tritiated thymidine incorporation were significantly increased in both T1DM patients and controls ($p < 0.001$ for all comparisons).
Both patients and controls had similar responses in terms of tritiated thymidine incorporation or IFN gamma production; there was no significant difference in responses to insulin peptides either with or without Ii-Key hybrids. However, some patients had strong responses to selected peptide hybrids and others had reduced responses, suggesting heterogeneity of the response, which could have masked the nature of the response. The basis for such differences in selected patients is the subject of continuing studies.
Conclusion: Ii-Key/MHC class II epitope hybrids are a novel reagent to analyzing T cell responses to antigens in autoimmune disease, where often the frequency of antigen-specific precursor cells in the blood is low. Our results indicate heterogeneity in the nature of the response in T1DM, whilst confirming that selected patients respond well to the Ii-Key hybrids.

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A novel approach to study T cell response to Ii-KEY/MHC class II epitope hybrid peptides in type 1 diabetes
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Background and Aims: Type 1 diabetes (T1DM) is the result of the immune-mediated destruction of pancreatic beta cells. T cell recognition of islet antigen(s) via MHC class has been postulated as a key mechanism in the induction of such immune response. It is unclear which autoantigen(s) and determinant(s) are targets of the autoimmune attack towards beta cells. A number of antigens have been associated with T1DM, insulin and insulin peptides being probably the most relevant ones, however a T cell assay for insulin/peptides with high sensitivity and specificity is still missing. A novel approach to study antigen presentation via HLA class II is represented by Ii protein associated with. It has been shown that a peptide of the Ii protein (Ii-key) enhances in vitro antigenic peptide presentation. These Ii-key peptides act at an allosteric site on MHC class II molecules to facilitate charging and presentation of peptides into the antigenic binding site. In previous studies it was demonstrated that Ii-key hybrids consisting of antigenic peptide-flexible-linker-Ii-Key peptide enhance presentation of antigenic peptides. The aim of this study was to apply this novel technique to establish the validity of Ii-Key/MHC class II insulin epitope hybrids for detecting anti-insulin responses in T1DM patients.
Materials and Methods: Insulin peptides shown to be recognized by T1DM CD4+ T cells were selected from data available in the literature. Sequences of the human insulin were obtained from Genebank. Predicted DRB1*0301 and DRB1*0401 epitopes were identified by application of the Rammensee SYFPEITH program (http://syfpeithi.bmi-heidelberg.com/Scripts/ MHC-Server.dll/EpPredict.htm) to the sequences of the experimentally characterized peptides containing DR-presented sequences. Within a homologous series, the longest and shortest hybrids were taken for initial synthesis plus the shortest peptide of 9 amino acids being considered to act as control. Five series (15 hybrids and five epitope-controls) were analyzed. The present study was carried out using mononuclear cells from 23 T1DM patients (18 males) (age range 7–41 years) with different disease duration including recent onset T1DM. Fresh peripheral blood mononuclear cells were prepared from heparinised blood using standard Ficoll Hypaque separation and cultured for 24 hrs at 37C for interferon gamma analysis using ELISPOT assays (BD Pharmingen) according to the manufacturer's instructions.
Results: Three patients (13%) showed strong activity with the longest hybrid of two different series and no activity with the shorter hybrids or all hybrids shorter than that one. A more potent activity was detected in presence of higher distance between the Ii-key moiety and the MHC class II epitope. Hybrids containing shorter spacer sequences were all less potent than hybrids with longer spacer sequence. Responder rates were lower in T1DM patients of longer disease duration than in patients with recent onset disease.
Conclusion: Data from this novel approach show that some hybrids, and not the epitope-peptide only, induce strong T cell reactivity in some patients with T1DM. The increased potency of MHC class II epitope presentation created by the Ii-key moiety in a hybrid should allow effective responses in those cases which are "low responder" to the epitope-peptide only. If these results are confirmed, they could offer the potential for a new diagnostic tool for studying T cell mediated immune responses in T1DM.
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OP 48

Resistin and visfatin

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Prodiabetogenic effect of transgenic resistin expression in the old spontaneously hypertensive rat

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Background and Aims: Resistin is a novel adipocytokine which has been shown to be involved in insulin resistance in obese mice as a possible link between obesity and type 2 diabetes. Recently these findings were questioned by findings that resistin mRNA expression in adipose tissue and plasma resistin levels were lower in subjects with type 2 diabetes and obese rodents and thus its biological role remains to be elucidated. We have previously shown that transgenic expression of resistin in the adipose tissue of spontaneously hypertensive rats (SHR), even without change in serum levels, was associated with increased serum free fatty acids, skeletal muscle triglyceride accumulation and resistance to insulin action. In this study we investigated if increased expression of resistin in adipose tissue has affects age-related change in glucose tolerance and insulin resistance of peripheral tissues in one year old transgenic SHR.
Materials and Methods: One year old male SHR expressing the mouse resistin gene under control of fat-specific aP2 promoter were used. Control group comprised age-matched genetically identical rats with absence of the transgene. All animals were fed a diet with 60% fructose for 2 weeks before the end of the study. Tissue sensitivity to insulin action was measured in vitro without or with insulin (250 μU/ml) according to basal and insulin-stimulated ^{14}C-U-glucose incorporation into muscle glycogen or adipose tissue lipids.
Results: One year old transgenic rats displayed higher body weight (389 ± 6 vs 370 ± 5 g, $p < 0.05$) and elevated epididymal fat pad weight (0.922 ± 0.035 vs 0.709 ± 0.043 g/100g BW, $p < 0.02$) compared to the control group. Serum triglyceride concentrations were increased before administration of high-fructose diet (0.82 ± 0.050 vs 0.63 ± 0.03 mmol/l, $p < 0.01$) and in both fasted (1.04 ± 0.07 vs 0.81 ± 0.03 mmol/l, $p < 0.01$) and postprandial state (1.99 ± 0.15 vs 1.34 ± 0.11 mmol/l, $p < 0.01$) after the fructose diet. The transgenic expression of resistin substantially impaired the tolerance to the oral glucose load (glycemia in 60 min.: 9.1 ± 0.4 vs 6.0 ± 0.1 mmol/l, $p < 0.05$; glycemia in 120 min.: 10.3 ± 1.6 vs 6.2 ± 0.18 mmol/l, $p < 0.02$; AUC_{0-120}: 1026 ± 131 vs 725 ± 14 mmol/l/120 min., $p < 0.02$) and increased hyperinsulinemia (339 ± 21 vs 270 ± 24 pmol/l, $p < 0.05$). Expression of transgenic resistin was associated with almost total adipose tissue resistance to insulin action measured in vitro according to ^{14}C-U-glucose incorporation into lipids during incubation without insulin (32.1 ± 4.0 vs 53.6 nmol//mg protein/2 hr, $p < 0.02$) or with insulin (48.6 ± 13.2 vs 130.3 ± 22.4 nmol//mg protein/2 hr, $p < 0.01$). On the other hand, in musculus soleus basal and insulin-stimulated glycogen synthesis were not different between SHR transgenic and control rats.
Conclusion: These results indicate that chronic transgenic expression of resistin gene was associated in one-year old animals with increased serum triglycerides, hyperinsulinemia, markedly impaired glucose tolerance and almost total insulin resistance of adipose tissue. Our data suggest possible involvement of resistin in age-induced development of insulin resistance and diabetes.
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Murine resistin triggers inflammatory responses in murine endothelioma (b.End.5) cells

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Background and Aims: Resistin, originally identified as an adipocyte-specific secretory factor (ADSF) implicated in insulin resistance, belongs to a newly discovered family of small cysteine-rich secretory proteins, called resistin-like molecules (RELMs) and found in inflammatory zones (FIZZ). Mice lacking resistin, *rstn (-/-)*, or expressing a dominant negative (hFc)-resistin fusion protein, exhibit improved glucose tolerance and insulin sensitivity when fed a high-fat diet, compared with wild type littermates. Human resistin, which shows significant sequence diversion from the murine form, induces endothelial expression of adhesion molecules and chemokines. This study investigated the inflammatory responses triggered by murine resistin and provides direct support for the concept of resistin-induced vascular dysfunction.
Materials and Methods: Murine endothelioma cells (b.End.5) (3 x 10^5) were incubated in DMEM medium, containing UltraGlutamine and penicillin/streptomycin (5000IU/ml), and with the addition of murine recombinant resistin (0–100 ng/ml, Biogenesis). Culture medium supernatants were collected (0–24 h) and JE/Monocyte Chemotactic Protein-1 (MCP-1) secretion assessed by solid phase sandwich ELISA (Biosource). Concentrations of cytokines, interleukin (IL)-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12p70, granulocyte macrophage colony stimulating factor (GM-CSF), interferon (IFN)-γ and tumour necrosis factor (TNF)-α were measured using a commercial array (Proteoplex™ Murine Cytokine Array, Merck). This array provides 16 sub-arrays, each containing quadruplicate spots of 10 antibodies, and positive and negative controls. Values represent mean±s.d. for numbers of independent experiments [n] performed, using quadruplicate wells in each experiment, or for array determinations reported; n.d. not detected.
Results: Secretion of JE/MCP-1 was essentially linear during incubation (0–24 h) in the presence or absence of resistin (7.5ng/ml). Significant increases in (24 h) increases in JE/MCP-1 secretion at were noted at 5 ng/ml (92.7 ± 16.0ng/mg cell protein [n=5] $p<0.02$ *versus* control 62.0 ± 18.9ng/mg cell protein [n=6]), 7.5ng/ml (117.3 ± 36.1ng/mg cell protein, [n=3], $p<0.02$) and 10ng/ml (124.2 ± 26.7ng/mg cell protein, [n=5] $p<0.001$) of murine resistin. At higher concentrations of resistin, secretion of MCP-1 declined to control levels. Resistin also induced increases in secretion of IL-6 (12.8 ± 0.97 ng/mg cell protein $p<0.001$*versus* control 9.4 ± 0.37ng/mg cell protein), IL-10 (1.76 ± 0.03ng/mg cell protein, $p<0.001$ *versus* control n.d.) and GM-CSF (0.69 ± 0.07ng/mg cell protein, $p<0.001$ *versus* control 0.13 ± 0.12ng/mg cell protein); all values mean±s.d. of quadruplicate array determinations; none of the other seven cytokines tested showed increased levels in the tissue culture medium.
Conclusion: Murine resistin induces specific and selective increases in cytokine secretion from b.End.5 endothelioma cells, suggesting that this molecule plays an inflammatory and potentially pro-atherogenic role during insulin resistance and (type 2) diabetes.
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Resistin is a pro-inflammatory cytokine targeting both human PBMC and adipose cells

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Background and Aims: Resistin, identified in murine and rodent adipose cells, was initially thought to be a link between obesity and insulin resistance in man. However, we and others were unable to determine resistin in human adipocytes although some reports claimed that it was expressed. In contrast, resistin is expressed in and secreted by human peripheral blood mononuclear cells (PBMC) where it can play an important role in inflammation. For instance, we have recently found that synovial fluid from patients with rheumatoid arthritis has markedly elevated resistin levels and recombinant resistin, like LPS, can up regulate resistin gene and protein expression in PBMC. Induction of resistin in human cells is dependent on the transcription factor C/EBPε.
Here we examined the effect of resistin on gene expression in PBMC as well as human adipose tissue and re-examined if human adipose cells express resistin or the necessary transcription factor C/EBPε.
Materials and Methods: Human PBMC and adipose tissue explants were cultured with resistin and LPS or resistin and TNF, respectively. Gene expression was assessed by real-time RT-PCR and secreted protein level was measured by ELISA. All three resistin PCR constructs, including reference Assay-on-Demand Hs00220767_m1 from Applied Biosystems, span the same exon 3/4 junction at position 242 in NM_020415.
Results: As expected, human PBMC express both resistin and C/EBPε. In contrast, human adipocytes do NOT express resistin or C/EBPε. Using three different RT-PCR constructs, resistin mRNA expression was examined in other samples like human muscle, subcutaneous and omental fat tissue biopsies and cells from synovial fluid with the same result.
However, both human adipose and PBMC are target cells for resistin. Both TNFα and resistin increased mRNA levels and secretion of several inflammatory modulators like IL-6, IL-8, IL-1β and MMP3 in cultured human adipose tissue. Interestingly, resistin did not affect markers of adipose differentiation, like aP2, C/EBPα and GLUT-4, while this was found with TNFα.

Conclusions:
1. Resistin and C/EBPε are NOT expressed in human adipose cells in contrast to human PBMC.
2. Resistin, like TNFα, increases cytokine expression in both human adipose tissue and PBMC.
3. Resistin seems to preferentially induce an inflammatory response in the adipose tissue and, in contrast to TNFα, does not alter markers of differentiation.

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Visfatin mRNA levels are increased in peripheral mononuclear cells from women with type 2 diabetes

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Background and Aims: Adipose tissue secretes several proteins (adipokines) that may influence insulin sensitivity and atherogenesis. Visfatin is a newly identified adipokine, highly expressed in human visceral fat. Visfatin's plasma levels are increased with expanding obesity and it is thought to promote accumulation of visceral fat, most propably acting through the insulin receptor pathway. We examined whether visfatin is expressed in human peripheral monocytes and whether its mRNA levels are altered in type 2 diabetes. We also examined its relationship to the mononuclear cell expression of TNFα and to indices of insulin resistance.
Materials and Methods: We studied 15 overweight women (BMI>25) with type 2 diabetes (DM2) and 27 healthy women with normal glucose tolerance [15 with BMI>25 (NGT-overw), and 12 with BMI<25 (NGT-lean), all premenopausal. We measured relative visfatin and TNFα mRNA levels in isolated blood monocytes, using a real-time quantitative RT-PCR assay (LightCycler, ROCHE) and human β-actin as a control gene. We also measured plasma TNFα and adiponectin levels with an Elisa kit (R&D Systems) and fasting and 2 hour post-OGTT glucose and insulin (RIA).
Results: Visfatin mRNA was easily detected in human mononuclear cells in both DM2 and control patients. Its relative mRNA levels were several-fold higher in DM2 compared to NGT-lean or NGT-overw controls (Table) and correlated overall significantly with BMI, waist circumference and the HOMA-IR index ($p<0.05$, $r=0.310$). Furthermore, visfatin mRNA levels correlated significantly with mononuclear cell TNFα mRNA levels as well as with circulating TNFα levels, while they correlated negatively with adiponectin levels.

	BMI kgr/m^2	Visfatin mRNA	TNFα mRNA	TNFα pg/ml	Adiponectin μg/ml
NGT-lean	21.8 ± 0.7	0.66 ± 0.32	0.03 ± 0.01	2.1 ± 0.2	15.5 ± 2.2
NGT-overw	32.3 ± 1.4*	0.63 ± 0.22	0.03 ± 0.01	2.2 ± 0.2	11.5 ± 1.8
DM2	34.3 ± 1.8*	2.05 ± 0.61*§	0.31 ± 0.16*§	3.3 ± 0.3*§	4.4 ± 1.2*§

*, $p<0.01$ vs NGT-lean; §, $p<0.01$ vs NGT-obese

Conclusion: Visfatin is expressed in human peripheral mononuclear cells and its expression is elevated in type 2 diabetes. This might contribute to the visceral fat accumulation and the atherogenic risk that characterizes type 2 diabetes.

PS 1
Genotype, insulin secretion and response to treatment

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Pharmacogenetic association of common haplotypes of the NIDDM1 gene (*CAPN10*) with weight gain and glycaemic response during 26 weeks of treatment with a PPAR-γ/α agonist in type 2 diabetic patients

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Background and Aims: The insulin sensitising effect of peroxisome proliferator-activated receptor-γ agonists has been established as a useful target in the treatment of insulin resistant type 2 diabetes. Variation within the calpain-10 gene (*CAPN10*) is associated with insulin resistance and an increased susceptibility for developing type 2 diabetes in several ethnic populations. The aim of the present study was to investigate if variation in the *CAPN10* was associated with differential clinical responses during an oral antidiabetic therapy in patients with type 2 diabetes.
Materials and Methods: 345 type 2 diabetic patients randomized to monotherapy with either 1 or 2 mg/day of the dual acting PPAR-α/γ agonist ragaglitazar in a double-blind, randomized, parallel, active-controlled (Glucophage 2000 mg or Diabeta 20 mg) trial of 52 weeks were included in the present study. The trial was stopped prematurely at 26 weeks, at which time the mean HbA1c % of the two treatment groups (1 and 2 mg/day) had dropped by approximately 1.1 HbA1c %. Haplotypes were generated (Genaissance Pharmaceuticals, Inc., New Haven, CT, USA) by genotyping (MALDI-TOF) ten single nucleotide polymorphisms covering the coding region and the 5'upstream region of the *CAPN10*. General linear regression models were run with Box-Cox transformed variables (HbA1c, weight gain and BMI) after 26 weeks of treatment as the endpoints, and with haplotypes h1, h2, h3, h4 and hx as the markers of interest, adjusting for age, sex, treatment, BMI, ethnicity, diastolic BP and the baseline level of the endpoint.
Results: Haplotype mapping classified the 345 type 2 diabetic patients into 5 different groups of common haplotypes: h1, 2111211122, 60%; h2, 1121111221, 9%; h3, 2112211222, 8%; h4, 2211222222, 8%; hx, 15%. A general test showed no difference between haplotype effects, but pair-wise comparisons revealed that patients with the h4 haplotype gained on average 2.7 kg ($p = 0.02$) of weight and 0.94 units of BMI ($p = 0.01$), whereas patients with the h2 haplotype dropped 0.38 HbA1c % points ($p = 0.02$) less than the hx reference haplotype.
Conclusion: The present study indicates that variation in the *CAPN10* gene may be involved in the glycaemic control and weight response during antidiabetic therapy with a PPARγ/α agonist in patients with type 2 diabetes. Further studies in type 2 diabetes patients of the *CAPN10* haplotypes during other antidiabetic therapies (ie insulin, metformin, sulfonylureas) are needed to confirm if the *CAPN10* gene is a general regulator of pharmacodynamic response to an antidiabetic treatment.

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Genotype and beta cell response to therapy in a large type 2 diabetic cohort

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Background and Aims: Identification of common genetic variants able to predict therapeutic response to currently available agents would revolutionise clinical care delivery. In the present study, we have examined two genetic variants implicated in type 2 diabetes susceptibility, in participants from the UKPDS study to establish whether genotype was related to response to therapy.
Materials and Methods: The UKPDS was a large, multi-centre, prospective, randomised, intervention trial of 5102 patients with newly diagnosed type 2 diabetes. Following a dietary run-in, patients unable to achieve fasting plasma glucose (fpg) <6 mmol/l (n=4209) were randomised to either diet alone (n=1138), aiming to remain free of diabetic symptoms and maintain-

ing a fpg of <15 mmol/l; or to sulphonylurea therapy (n=1573) or a basal insulin supplement (n=1156), with intent to maintain a fpg <6 mmol/l. Overweight patients could also be randomised to metformin (n=342). Genotyping, by allelic discrimination, of the *PPARG* Pro12Ala variant and *INS* VNTR class (inferred by genotyping the -23 *HphI* variant) was carried out on DNA available from 4139 participants. HOMA derived beta cell function was calculated in the 2472 participants with complete data. Two measures of treatment response were examined; (i) change in %B in the first year following randomisation, analysed using multiple regression, after adjustment for a range of factors including therapy allocation, sex, ethnicity and GAD antibody status and ii) time to halving of %B (or requirement for insulin therapy) from one year after randomisation, analysed using a Cox proportional hazards model, again after adjustment.
Results: *PPARG* Pro12Ala genotypes were distributed as Pro/Pro, 82.4%; Pro/Ala, 16.7%; Ala/Ala, 0.9%. Distribution of the *INS* VNTR was: Class I/I, 48.4%; Class I/III, 38.7%; Class III/III, 12.9%. All alleles were in Hardy Weinberg equilibrium. With respect to genotype, no significant change in beta cell function was observed in either a univariate regression model (*PPARG*, n=1804; *INS* VNTR, n=1805) or in a multivariate model adjusted for therapy allocation, gender, ethnicity, beta cell function at randomisation, antibody status, BMI and lipids (*PPARG*, n=1471; *INS* VNTR, n=1469). In a Cox proportional hazards model, adjusted for the same covariates, there was no significant effect of genotype on time to halving of %B (*PPARG*, n=1383; *INS* VNTR, n=1384). When these same measures were examined in groups stratified by randomised therapy, again no differences in %B were seen by genotype. For example, there was no difference in mean change in %B between genotypes in overweight patients randomised to diet (*PPARG* Pro/Pro -12.2%, Pro/Ala -13.5%, Ala/Ala -17.8%, p >0.15, adjusted; *INS* VNTR Class I/I -14.3%, Class I/III -10.7%, Class III/III -11.9%, p >0.15, adjusted).
Conclusions: In the current study, there was no relationship between beta cell responses to diet, sulphonylurea or metformin therapy and common genetic variation in the *PPARG* or *INS* genes.
Support: Diabetes UK

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The A98V polymorphism in the *HNF-1a* gene predicts change in HbA1c over time in patients with newly diagnosed type 2 diabetes
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Background and aim: Type 2 diabetes (T2D) is a chronic multifactorial disease, with a progressive rise in blood glucose and HbA1c over time, despite an initial improvement. The increase in HbA1c has been ascribed to deterioration of β-cell function. While many studies have focused on genetic causes of T2D, few studies have addressed the question whether genetic variants influence the course of the disease. Since inactivating mutations in the glucokinase gene (MODY2) prevent their carriers from progression of the disease, we set out to test the hypothesis whether mutations in *HNF1*α or *Kir 6.2*, which also are associated with impaired β cell function, influence the course of the disease.
Materials and methods: 848 T2D patients from the local diabetes registry in southern Sweden were followed with repeated HbA1c measurements during 8 years after diagnosis. All subjects were genotyped for the three common variants in the *HNF1-*α gene, -I27L, -A98V, and -S487N and E23K in the *Kir 6.2* KCNJ11 gene. The relation between genotype and changes in HbA1c during the follow up period adjusted for age, sex and BMI at diagnosis were studied using a generalized estimating equation procedure.
Results: HbA1c showed significant improvement after one year of diagnosis, from 6.70 ± 2.32 to 6.16 ± 1.57 (P=<0.0001). However, thereafter, HbA1c showed a progressive rise, from 6.16 ± 1.57 to 6.69 ± 1.71, during 7 years of follow up (P=0.0026). There was a significant increase in HbA1c over time in AA-genotype carriers of HNF1α A98V (r = 0.1188591; CI 0.079097–0.158621, P<0.001), but not in -AV or -VV- carriers. No significant increase in HbA1c over time were seen in carriers of the common variants in *HNF1*α- I27L, - S487N or *Kir 6.2* KCNJ11.
Conclusions: We observed the expected rise in HbA1c over time in patients with newly diagnosed T2D. However, there was no increase in HbA1c in carriers of the A98V or V98V genotypes of HNF1α. As these genotypes have been associated with decreased transcriptional activity and decreased insulin secretion, the question rises whether a small "metabolic block" in the islets can prevent them from deleterious consequences of glucotoxicity.

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Calpain 10 and the function of isolated type 2 diabetic and non-diabetic pancreatic islets
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Background and Aims: Calpain 10 (CAPN10) is the first type 2 diabetes (T2D) susceptibility gene that has been identified by a genome scan and the G allele of SNP 43 has been associated with decreased CAPN10 mRNA in skeletal muscle and insulin resistance. Recent evidence has shown that CAPN10 is present in human pancreatic islets, and that it may act as a regulator of insulin secretion in beta-cell lines and rodent islets.
Materials and Methods: We prepared human islets by enzymatic digestion and gradient separation from the pancreas of T2D (n: 11; age: 64.1 ± 8.3 yrs; BMI: 27.1 ± 2.9 kg/m^2) and non-diabetic (n: 37; age: 53.4 ± 16.9 yrs; BMI: 24.9 ± 3.7 kg/m^2) organ donors, and genetic, molecular and functional studies were performed to assess the relationships between CAPN10 SNP-43 genotype, CAPN10 mRNA expression and insulin secretion.
Results: CAPN10 mRNA expression (after normalization for cyclophylin and GAD mRNA expression) was significantly (p<0.01) higher in diabetic (T2DI, 1.5 ± 0.8) than in non-diabetic (NDI, 0.8 ± 0.4) islets. No significant difference in GG, GA and AA genotype distribution was observed between T2DI and NDI, i.e. 36% vs. 54%, 55% vs 32%, and 9% vs 14%, respectively. The CAPN10 SNP43 genotype did not influence CAPN10 mRNA levels in NDI (GG vs GA+AA: 0.7 ± 0.4 vs 0.8 ± 0.3), nor in T2DI (1.3 ± 0.7 vs 1.6 ± 0.9). In the NDI group, glucose-stimulated insulin secretion was positively (p<0.05) correlated with CAPN10 mRNA expression, but not affected by the SNP43 genotype. The correlation between CAPN10 mRNA expression and insulin secretion was lost in T2DI.
Conclusions: These results show that: 1) in type 2 diabetic islets, CAPN10 mRNA expression is higher than in non-diabetic islets, but uninfluenced by the SNP 43 genotype; 2) in non-diabetic islets, CAPN10 mRNA expression correlates with glucose-stimulated insulin secretion. The present findings that CAPN10 mRNA expression is increased in type 2 diabetic human islets, together with our previous results showing decreased expression in skeletal muscle of insulin resistant individuals, suggest a dual role for CAPN10 in the regulation of insulin secretion and insulin action in humans.

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PGC-1 alpha genotype affects PGC-1 alpha mRNA expression and glucose-stimulated secretion in isolated human pancreatic islets
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Background and Aims: Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 alpha) facilitates PPAR-gamma and PPAR-alpha mediated gene transcription. Recent genetic studies have implicated PGC-1 alpha in the defects of oxidative phosphorylation in muscle of diabetic and pre-diabetic individuals. PGC-1 alpha also is expressed in beta-cells where it may contribute in the regulation of ATP and therefore be implicated in the regulation of insulin secretion in response to glucose. However, no information is available on the expression and function of PGC-1 alpha in human pancreatic β-cells. For this purpose, we have examined the influence of genetic and environmental factors on the expression of PGC-1 alpha in human pancreatic islets.
Materials and Methods: We determined DNA polymorphism (DNA extraction and allelic discrimination with ABI 7900) and mRNA expression (quantitative Real Time RT-PCR) from 34 pancreases of non-diabetic (Ctrl) (age: 54.5 ± 21.5 yrs, gender: 14/20 M/F, BMI: 24.2 ± 2.4 Kg/m^2) and 12 pancreases of Type 2 diabetic multiorgan donors (T2DM- 66.7 ± 8.3 yrs, 6/6 M/F, 27.0 ± 3.4 Kg/m^2). These data have been related to glucose-stimulated insulin release.
Results: The PGC-1 alpha *Gly482Ser* polymorphism had a significant effect on islet PGC-1 alpha mRNA expression both in Ctrl and T2DM. Carriers of more *Ser* alleles (*Gly/Ser* + *Ser/Ser*) had lower PGC-1 alpha mRNA levels compared with *Gly/Gly* genotype (2.94 ± 0.77 vs. 23.8 ± 4.4 optical density units, OD; p< 0.01). Glucose (16.7 mM) mediated insulin release (Stimulation index: fold increase over baseline) was significantly reduced in *Gly/Ser* + *Ser/Ser* polymorphic islets as compared to *Gly/Gly* (1.61 ± 0.18 vs., 2.05 ± 0.17; p< 0.05). Age was inversely related to both PGC-1 alpha expres-

sion (r= -0.34; p<0.05) and insulin release (r= -0.52; p<0.01). PGC-1 alpha expression was lower in T2DM islets as compared to Ctrl (1.10 ± 0.63 vs. 15.0 ± 3.0; p < 0.01). Insulin release from T2DM islets, also was reduced (SI: 1.25 ± 0.12 vs. 2.1 ± 0.1; p< 0.01).
Conclusions: PGC-1 alpha is expressed in human islets with a degree of expression that is affected by genotype and age. The reduction of PGC-1 alpha expression is associated with lower glucose-mediated insulin release. Finally, a significant reduction of PGC-1 alpha was found in islets from T2DM. We conclude that PGC-1 alpha may be a physiological modulator of human islet function.

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The role of nicotinamide nucleotide transhydrogenase in insulin secretion and impaired glucose tolerance
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Background and Aims: Insulin release from pancreatic β-cells is regulated by glucose metabolism. When plasma glucose levels rise, increased ATP generation, predominantly in the mitochondria, leads to closure of ATP-sensitive potassium (K_{ATP}) channels. This results in membrane depolarisation, activation of voltage-gated Ca^{2+} channels, influx of Ca^{2+} and exocytosis of insulin-containing vesicles. K_{ATP} channels in the β-cells of the inbred mouse strain, C57BL/6J, show impaired closure in response to glucose metabolism. However, they retain normal ATP sensitivity indicative of a defect further upstream perhaps at the level of β-cell metabolism. The glucose intolerance and reduced insulin secretion in these mice result in a phenotype reminiscent of human type-2 diabetes.
Through QTL mapping, we identified Nicotinamide Nucleotide Transhydrogenase (*Nnt)* as a strong candidate gene for the glucose intolerant phenotype in C57BL/6J mice. We also showed that C57BL/6J mice have a multi-exon deletion in *Nnt*. *Nnt* is a nuclear encoded mitochondrial gene that functions as a redox-driven proton pump, catalysing the reversible reduction of $NADP^+$ by NADH and conversion of NADH to NAD^+.
Materials and Methods: To investigate the role of *Nnt* more closely, we used siRNA to knock down expression of *Nnt* in the insulin-secreting cell line MIN6. Intracellular calcium and insulin secretion were measured in response to increasing extracellular glucose using Fura-2 imaging and insulin ELISA, respectively. We also identified two ENU-induced point mutations in exons 2 and 14 of the gene using the Harwell ENU-DNA archive. These mutants were recovered as live mice by IVF, and the progeny were intercrossed to produce mice that are homozygous for the mutations. Intraperitoneal glucose tolerance tests (IPGTT), under local anaesthetic, at 12 and 16 weeks of age were used to measure changes in glucose and insulin secretion following glucose challenge.
Results: Insulin secretion from MIN6 cells transfected with nonsense siRNA or *Nnt* siRNA in response to external glucose (10 mM) was substantially reduced (12.5 ± 1.8ng/ml for *Nnt* siRNA (n=6) compared to 34.7 ± 0.7 ng/ml for nonsense siRNA (n=6)). Likewise, the glucose-dependent increase in $[Ca^{2+}]_i$ was dramatically decreased (87.9 ± 13.6 nM, *Nnt* siRNA (n=21) compared to 180.4 ± 11.6 nM, nonsense siRNA (n=18)). IPGTT revealed that both heterozygous and homozygous males and female exon 2 *Nnt* mutants were significantly less glucose tolerant than their wild-type littermates at both 12 and 16 weeks. Heterozygotes and homozygotes also secreted substantially less insulin over a 30 minute period following a glucose challenge. Similar results were found for exon 14 *Nnt* mutants.
Conclusion: These results establish a functional linkage between *Nnt* and both glucose intolerance and reduced insulin secretion. We hypothesise that *Nnt* knockdown impairs β-cell mitochondrial metabolism leading to less ATP production, and thereby lowered K_{ATP} channel activity. Consequently, glucose-dependent β-cell electrical activity and insulin secretion are impaired.

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Marked jump in incidence of childhood onset diabetes in Norway after ten years of stable incidence: prospective nationwide study 1999–2003
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Background and Aims: The incidence of childhood onset type 1 diabetes was relatively stable at 22.5 per 100 000 in Norway in the period 1989–1998. The aim was to describe the incidence of childhood onset diabetes in Norway for the period 1999–2003.
Materials and Methods: New cases of insulin-requiring diabetes diagnosed before the age of 15 years were reported prospectively nationwide as part of the EURODIAB incidence study. Person-years were estimated by the mean population in 5-year age-groups for each calendar year based on information from Statistics Norway. Incidence rates per 100 000 person-years were computed and 95% confidence intervals estimated based on the Poisson-distribution and using a log transformation.
Results: In this preliminary report, the total number of new cases registered 1999–2003 was 1260 (mean of 252 per year), resulting in an overall incidence of 27.9 (95% CI: 26.4–29.5). The incidence was relatively stable for the current study period. For the age-groups 0–4, 5–9 and 10–14, the incidence was 17.1, 30.6 and 36.0 per 100 000 person-years. The incidence was 29.2 for boys and 26.7 per 100 000 person-years for girls.
Conclusion: Our data indicated a marked jump in incidence from 22.3 in the previous ten-year study period (1989–1998) to 27.9 per 100 000 person-years in the current period (1999–2003), representing a 25% increase over a very short period. The causes for this rapid increase are unknown, but should be the focus on future research.

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The incidence of childhood type 1 diabetes in Romania. A ten-year follow-up
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Background and Aims: The incidence of childhood type 1 diabetes mellitus (DM) in Romania is low, but with an ascending trend. The aim of this paper is to assess the time trends in the incidence of childhood DM and to outline some epidemiological features concerning the gender and ethnic distribution of the patients.
Materials and Methods: Between 1995 and 2004 all newly diagnosed cases of type 1 DM in the age group 0–14 years have been recorded. The main data source for newly diagnosed type 1 DM in children is represented by a group of pediatricians and diabetologists constituted in ONROCAD (Romanian acronym for Romanian National Organization for the Protection of Children and Adolescents with Diabetes). For each case they filled in a chart containing: patient's name, address, ethnicity, date of birth, age at diagnosis, date of first insulin injection, as well as the name and address of the treating physician. A second source of data is represented by the Clinical Center "Cristian Serban" for Evaluation and Rehabilitation for Children and Adolescents, Buzias, unique in Romania, where a large number of children with type 1 DM from all over the country are admitted, evaluated, educated and treated. Data were collected, recorded electronically and analyzed and a National Type 1 Diabetes Register was issued yearly.
Results: General, as well as gender and ethnic specific incidence of type 1 DM in children (0–14 years) between 1995 and 2004 is presented in Table 1.

Table 1.

General incidence*		1995 3.57	1996 3.91	1997 4.08	1998 4.10	1999 4.97	2000 5.05	2001 5.66	2002 4.88	2003 5.47	2004 5.36
Gender*	G	–	3.96	4.74	4.34	5.23	4.94	5.04	4.34	5.41	4.91
	B	–	3.88	3.99	3.72	4.56	4.15	5.15	5.34	5.52	6.26
Ethnicity*	R	–	–	–	3.90	4.57	4.82	5.53	4.64	5.23	5.38
	H	–	–	–	7.02	8.98	7.88	7.19	5.91	11.63	9.77

G= girls; B= boys; R= Romanian; H= Hungarian; *no.of cases/100,000

Several remarks can be made:
a) incidence increased with 58.54% between 1995 and 2001 (up to 1995 general incidence was unknown) above 5/100,000 and remained constant thereafter (except for 2002); b) until 2002 type 1 DM incidence was higher in girls, thereafter the gender-specific incidence has reversed; c) regarding ethnicity, the incidence in Romanians (the majority population) is clearly lower than that in Hungarians (the largest minority group); the only explanation for this observation resides in the different genetic background, supported by the fact that the type 1 DM incidence in children of Hungarian nationality is similar to that encountered in the neighbor Hungary; no difference in the environmental factors could be found in the two communities.
Conclusions: In Romania, the general incidence of type 1 DM in age group 0–14 years has gradually increased between 1995 and 2001, and remained constant afterwards. In the past 5 years type 1 DM was more frequently diagnosed in boys; children from the Hungarian minority are more frequently affected than those from the predominant Romanian population.
The years to come will bring new data on the evolution trends of childhood type 1 DM epidemiology in Romania.

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The incidence of childhood type 1 diabetes in the Cracow region during 1987–2003

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Background and Aims: The results of the epidemiological studies reveal a huge variability in the incidence in childhood diabetes mellitus worldwide. The aim of the study was to determine the incidence of type 1 diabetes in children aged 0–14 years between 1987 and 2003 living in Cracow region. During 17 years of the study period 379 children (201 boys, 178 girls) with newly diagnosed type 1 diabetes were identified. The study was based on prospective registry as a part of EURODIAB ACE study and within a project of Ministry of Health from 1998.
Results: the annual crude incidence rates in children aged 0–14 years in both genders in the Cracow region were following.
In 1987 – 5,3; 1988 – 7,0; 1989 – 4,6; 1990 – 7,2; 1991 – 6,2; 1992 – 7,4; 1993 – 7,9; 1994 – 6,9; 1995 – 6,3; 1996 – 10,6; 1997 – 12,6; 1998 – 13,0; 1999 – 15,2; 2000 – 5,5; 2001 – 15,8; 2002 – 10,3; 2003 – 13,7.
The analysis of the trend showed a significant increase in incidence in children; the greatest increase of incidence was observed in 5–9 years subgroup of children. The lowest incidence rates were noted in children aged 0–4 years and in children aged 5–9 and 10–14 years the incidence rates were similar. Neither girls nor boys showed a significant predominance.
The incidence rates for children living in the cities were significantly higher compared to those living in villages. The rising trend in the incidence was greater in the urban in comparison to rural region.
The incidence rates exceeding 10/100,000 per year observed since 1996 have placed Cracow region to the group of areas with high risk of type 1 diabetes mellitus.

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Incidence of type 1 and type 2 diabetes in adults aged 30–49 years: the population-based registry in the province of Turin, Italy

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Background and Aims: Incidence of type 1 diabetes is considered very low in adults, but no population-based study has been performed in Mediterranean countries. Classification based on clinical presentation can underestimate the true number of cases arising among adults, many being misclassified as type 2 diabetes. The presence of markers of β-cell autoimmunity, such as islet cell antibodies (ICA) and glutamic acid decarboxylase (GADA), allows the identification of type 1 diabetes, independently of age and clinical presentation at disease onset. In Northern Italy, the incidence rates among persons up to 29 years of age have been estimated by the Registry of the Province of Turin, which has achieved high estimated completeness of ascertainment for both children and young adults. In this report, we aimed to extend the study base of the registry to persons aged 30–49 years in the period 1999–2001 and to estimate the incidence rates of type 1 and type 2 diabetes, by using both clinical and immunological features of incident cases.
Materials and Methods: The study-base is the population 30–49 years of age of the Province of Turin, period 1999–2001, identified through two independent sources of ascertainment. Diagnosis of type 1 diabetes was based on permanent insulin treatment or a fasting C-peptide level ≤0.20 nmol/l or ICA/GADA positivities. In 2003–2004, clinical charts of all non-tested subjects at diabetes onset were ascertained to assess treatment.
Results: 1135 incident cases of diabetes have been identified (completeness of ascertainment 99%, incidence of 58.0/100,000 person-years, 95% CI 54.7–61.5), with 5.5-fold higher risk in the age-group 45–49 yrs than in age-group 30–34 yrs (95% CI 4.47–6.79). Out of 617 tested subjects at diabetes onset, 98 (15.9%) were ICA and/or GADA positive. Only 40 out of non-tested subjects were insulin-treated since onset of disease. Incidence of type 1 diabetes was 7.3/100,000 (95% CI 6.2-8.6). Higher risk in men than in women was found for both type 1 (RR=1.70, 95% CI 1.21–2.38) and type 2 diabetes (RR 2.10, 95% CI 1.84–2.40). Decreasing ratios of type 1 to all new cases of diabetes with increasing age were found in both sexes: 30% in the age group 30–34 years, 19% in the age group 35–39 years, 11% in the age group 40–44 years and 8% in the age group 45–49 years. In logistic regression analysis, normal-weight subjects with fasting plasma C-peptide values <0.60 nmol/l had 6-fold higher risk of autoimmune diabetes than subjects with both BMI ≥26 mg/kg^2 and C peptide ≥0.60 nmol/l, independently of age and sex.
Conclusion: This population-based study shows that: 1) the risk of adults for type 1 diabetes – defined as persistent insulin treatment or positive markers of β-cell autoimmunity – is similar to that found in the same area for persons of post-pubertal age; 2) the risks for both type 1 and type 2 diabetes are twofold higher in men than in women in every age group; 3) persons of normal weight with fasting C-peptide levels below 0.60 nmol/l have a sixfold higher likelihood of being positive for ICA or GADA than all other persons; and 4) the incidence of type 2 diabetes was 50.7/100 000 person-years and 7-fold higher in age-group 45–49 yrs than in age-group 30–34 yrs. We propose that geographical differences in the incidence of childhood diabetes are due at least in part to a persistent high risk in adults living in areas of lower risk.

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Seasonality of birth and age at onset for diabetes in Yorkshire, UK

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Background and Aims: Rates of childhood diabetes continue to rise, particularly in the very young. The rapidity of this increase supports an environmental aetiology and the specific effect in young children suggests environmental factors operating around the time of birth may influence onset. Early exposure to infections has been suggested as being protective for childhood diabetes and infections are known to vary cyclically with season. We investigated seasonality of month of birth of children who developed diabetes looking specifically for any age differences, to test the hypothesis that effects may be seen most strongly in the youngest children.
Materials and Methods: Details of each child's month of birth were extracted from the Yorkshire Register of Diabetes in Children and Young People for those diagnosed with Type 1 diabetes under the age of 15 between 1978–2003, resident at the time of diagnosis within the Yorkshire in the north of the UK. All children were analysed together (0–14s) and separately by 5-year age groups (0–4, 5–9, 10–14 years). Seasonal variation was tested using Walter and Elwood's test and logistic regression including a periodic function of month of birth as a covariate, allowing for a variable population at risk using data derived from the number of births in England and Wales.
Results: 3204 patients in total were identified, with 681, 1077 and 1446 aged 0–4, 5–9 and 10–14 years at diagnosis, respectively. No significant evidence of seasonality was exhibited for all children combined (p=0.29), although a modest peak and trough were apparent in the summer and winter months, respectively. Subgroup analysis by age revealed that significant cyclical

variation only existed for patients aged 0–4 years (p=0.02), with the maximum and minimum predicted rate occurring in early June and early December, respectively. Results for 5–9 and 10–14 year olds were non-significant (p=0.21 and p=0.89, respectively). We examined the effect of censoring by year of birth and found similar results, although for 5–9 year olds there was some marginal evidence of seasonality (p=0.06).
Conclusions: Our findings suggest that seasonal environmental factors operating around the time of birth are not associated with the totality of childhood diabetes but appear to be restricted to those diagnosed under 5 years of age. Children born in early winter are more likely to be exposed to a higher level and range of infections immediately after birth than those born in early summer, therefore developing their immune system much more quickly. The hygiene hypothesis suggests that exposure in early life to infections may reduce the risk of abnormal immune reactions and subsequent disease: our findings are therefore consistent with this theory. The marked presence of seasonality by month of birth for those diagnosed under the age of five suggests that infectious exposure may play an important role in the development of diabetes, either around the time of birth or during the antenatal period.

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Is earlier age at onset of insulin-treated diabetes associated with higher body mass index?
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Background and Aims: It has been hypothesized that type 1 diabetes presents at an earlier age in heavier children, and an inverse relationship between age at onset and body mass index (BMI) at diagnosis has been described in a Caucasian population. With the incidence of childhood insulin-treated diabetes on the rise in young children, it has been suggested that obesity could be a contributing factor. Our aim was to determine if earlier presentation of insulin-treated diabetes is associated with increased BMI at onset in White and Black children.
Materials and Methods: All children <19 years of age, with residence in Allegheny County, Pennsylvania, diagnosed with insulin-treated diabetes at Children's Hospital of Pittsburgh between January 1995 and December 2002, were included (n=351). Data were obtained from retrospective review of medical records. Children were grouped into age-at-diagnosis tertiales. BMI Standard deviation scores (SDS) were calculated.
Results: Of these children, 70 (20%) were Blacks and 281 (80%) Whites; 174 (49.5%) were females and 177 (50.5%) males. The prevalence of overweight (BMI ≥ 85thile) at diagnosis was 5.8%, 25.2% and 42.3% in the age groups 0–6, 7–12, and 13–18 years respectively (p=0.0005).
BMI-SDS was lower in those who developed diabetes at a younger age (0–6 years, -0.884 ± 1.517; 7–12 years, -0.030 ± 1.623 and 13–18 years, 0.551 ± 1.551; p=0.0005). There was a positive correlation for BMI-SDS with age at diagnosis (r=0.30, p=0.0005). which remained when males and females, Blacks and Whites were analyzed separately (males r = 0.24, p = 0.002; females r = 0.38, p= 0.0005; Blacks r=0.32, p=0.011; Whites r=0.29, p=0.0005).
Conclusion: These results suggest that age at presentation of diabetes is positively associated with obesity both in Whites and Blacks, different from what has been described in other populations.
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Positive correlation between socio-economic status and estimated worldwide occurrence of type 1 diabetes and bronchial asthma
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Background and Aims: The atopic disorders, such as asthma, eczema and hay fever, and type 1 diabetes are immune-mediated diseases characterised by a rising prevalence beginning from the second half of the twentieth century, mainly in the affluent countries. The analysis of published data on the prevalence of asthma symptoms and the incidence of type 1 diabetes in both European and extra-European children showed a strong positive association between the occurrence of the two diseases at the population level. The association between occurrence of asthma and type 1 diabetes at the population level could be explained by altered environmental and lifestyle conditions, frequently related to socio-economic differences. To investigate the relationship between the incidence of these immunologic diseases and the economic status, we calculated the correlation between the occurrence of type 1 diabetes and bronchial asthma, the gross national product and the infantile mortality, in several European and extra-European countries.
Materials and Methods: Data for type 1 diabetes incidence among children aged 0–15 years were derived from childhood diabetes registers. Data for asthma prevalence were obtained from the International Study of Asthma and Allergies in Childhood (ISAAC), an epidemiological survey among children aged 13–14 years conducted in 1994 and 1995. The gross national products (GNP) per capita, expressed in United States Dollars (USD), and data on infantile mortality were available from The World Health Report 1995 by the World Health Organization. The Spearman correlation coefficients (r_{sp}) between the occurrences of the two diseases, the gross national products and the infantile mortality rates were calculated; a significant relationship between the variables was considered for pairs with p values below 0.05.
Results: GNP was positively correlated with the incidence of type 1 diabetes and with symptoms of asthma in European (r_{sp}: 0.53 and 0.69; $p = 0.001$ and $p < 0.0001$, respectively) and extra-European countries (r_{sp}: 0.44 and 0.46; $p = 0.04$ for both diseases). Infantile mortality rate was inversely correlated with GNP and with the occurrences of the two diseases in Europe (r_{sp}: - 0.66, $p < 0.0001$ for type 1 diabetes; r_{sp}: - 0.51, $p = 0.01$ for asthma). In extra-European countries a significant relationship was found between infantile mortality and asthma (r_{sp}: - 0.46; $p = 0.03$); a trend towards a negative correlation between infantile mortality and type 1 diabetes was also found, although no statistical significance was reached (r_{sp}: - 0.21; $p = 0.31$).
Conclusion: Our analysis indicates that type 1 diabetes and asthma symptoms are positively associated with the gross national product at the population level. Similarly, countries with low infantile mortality rates tend to have a higher incidence of these immune-mediated diseases. The most likely explanation is that a high socio-economic status implies a reduced or delayed exposure to infectious agents which in turn causes an insufficient stimulation of regulatory T cells and facilitates the onset of immune-mediated disorders.

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Socio-economic conditions and the risk of childhood type 1 diabetes mellitus in Germany
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Background and Aims: The incidence of type 1 diabetes has been shown to be increasing worldwide. The aetiology of the disease, however, is still unknown. It is believed that environmental exposures trigger a T-cell mediated autoimmune process in genetically susceptible individuals. Aim was to analyse associations between socio-economic conditions and the incidence of type 1 diabetes in an ecological study using aggregated spatial data.
Materials and Methods: For the period 1996–2000 data on type 1 diabetes incidence in children under 15 years of age on district level were taken from the diabetes register of North Rhine-Westphalia, the German federal state with the largest populations. Case registration has been shown to be 96% complete. Data on socio-economic conditions were obtained from the North Rhine-Westphalian statistical office. Socio-economic indicators used were population density, proportion of foreign population, measures of school education (secondary education vs. grammar/high school degree), vocational training (learned profession vs. university degree) and income (household income per person, income <1000 DM vs. ≥3000 DM), quotas of unemployment and social welfare recipients, proportions of single parents and size of living space. Spearman's rank coefficient (r_s) and usual Poisson regression analysis were used to assess associations between socio-economic conditions and type 1 diabetes risk. Possible unstructured and autocorrelated spatial variation of type 1 diabetes incidence was taken into account by applying Poisson random effects models and conditionally autoregressive (CAR) Poisson models using a Bayesian approach.
Results: Type 1 diabetes incidence was significantly associated with population density, proportion of foreign population, household income and income ratio (r_s = –.38, –0.46, –0.56 and 0.63, respectively). In Poisson regression analysis, significant associations with type 1-diabetes incidence were found for all socio-economic indicators, with the exception of living space and single parents. Observed associations were strongest for population density, proportion of foreign population, indicators of income, and education and vocational training. A one SD increase in population density, proportion of foreign population or household income was associated with a 7–10% decrease in diabetes incidence, a one SD increase in income ratio or measures of education and vocational training with a 6–10% increase in incidence. Poisson random effects and CAR models accounting for spatial

variation of incidence gave almost the same results. In multivariate analysis only income indicators remained significantly associated with the diabetes risk.
Conclusion: In this ecological study the incidence of type 1 diabetes was higher in economically and educationally deprived as well as less densely populated geographical areas. The observed associations were not affected by unstructured or autocorrelated spatial variation. The inverse association between type 1 diabetes incidence and population density is concordant with the so-called "hygiene hypothesis". The observed ecological associations, however, need to be confirmed by studies on an individual level. In particular, causal factors acting behind the socio-economic indicators have to be identified.
The study was supported by the Deutsche Forschungsgemeinschaft.

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Genetics of diabetes: vitamin D related genes and methodology

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CYP2R1 gene polymorphism in type 1 diabetes mellitus
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Background and Aims: The vitamin D (VD) system has been implicated in type 1 diabetes (T1D) by epidemiological and immune intervention studies as well as gene polymorphisms of the vitamin D receptor, CYP27B1, vitamin binding protein (DBP) and CYP24 where associations have been described.
The enzyme CYP2R1 catalyses the first step of the activation of VD. We therefore analyzed a novel CYP2R1 hydroxylase polymorphism in T1D families and correlated the variants with 25-OH- and 1,25-OH-VD serum levels in T1D patients.
Materials and Methods: Five polymorphisms rs12794714, rs12577735, rs12360784, rs110233376 and rs10741657 were genotyped in a set of 100 T1D patients and 100 healthy controls. Polymorphisms, which were prevalent in all three variants, were further investigated in simplex T1D families. One hundred three (429 subjects) and 184 (552 subjects) families were genotyped for the rs12794714 and rs10741657 polymorphisms, respectively. 25-OH VD and 1,25-OH VD were measured in 106 and 101 T1D patients. Additionally RNA expression studies were performed in 20 T1D patients by quantitative real-time PCR.
Results: Analysis of the rs10741657 polymorphism showed that the allele G was significantly more often transmitted to affected offspring (95 times vs. 56 times, p=0.0031). However no association was observed for rs12794714 (p=0.4094) nor any correlation between these polymorphisms and 25-OH-VD, 1,25-OH- VD levels or RNA expression rate.
Conclusion: Our findings reveal an association of the CYP2R1 polymorphism rs10741657-G with T1D. However its functional role needs to be further elucidated. At present we can not explain it with a possible influence either on VD levels or on the RNA expression. The CYP2R1 variant may interact with other genetic components of the VD system in the genetic susceptibility to T1D.
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Vitamin D receptor gene polymorphism in Southern Chinese adults with latent autoimmune diabetes (LADA)
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Background and Aims: Vitamin D (Vit D) has important immunomodulatory effects, supported by Vit D administration prevented the development of autoimmune diabetes in NOD mice. It has been reported that Vit D receptor (VDR) gene polymorphism was associated with T1DM in several populations. Latent autoimmune diabetes in adulthood (LADA) is a disease characterized by islet autoimmunity. The study was to investigate the relationships between the VDR gene polymorphism (FokI) and LADA in Chinese, and the titer of glutamic acid decarboxylase antibody (GAD-Ab) in diabetes.
Materials and Methods: The 166 normal controls, 115 LADA, 85 T1DM and 140 T2DM patients, were recruited from unrelated Southern Chinese. All subjects were genotyped using PCR-RFLP for FokI restriction site in the VDR gene. The titers of GAD-Ab were detected using radioligand assay, which was evaluated to be efficient in Diabetes Autoantibody Standardization Program (DASP 2003). This was a cross-sectional, case control study.
Results: (1) The frequency of VDR FokI FF, Ff and ff genotypes was 38.6%, 44.6%, and 16.9% respectively in the controls, which was different from the frequency of genotype in Northern Chinese controls (50.0%, 42.7% and 7.3%, P<0.05) and Japanese controls (13%, 55% and 31%,P<0.05). (2) The distribution of VDR FokI FF, Ff and ff genotypes was different among diabetes types (27.8%, 42.6% and 29.6% in LADA; 21.2%, 57.6% and 21.2% in T1DM; 21.4%, 59.3% and 19.3% in T2DM, respectively) (P<0.05). Compared with the controls, the frequency of FF genotype decreased and ff genotype increased in LADA (P<0.05). (3) The titer of GAD-Ab was the

highest in VDR ff genotype, followed by Ff genotype, and FF genotype in LADA patients (1.000, 0.227 and 0.092, respectively). No matter what the cut-off points of GAD-Ab titer were 0.2, 0.5 or 0.8, the distribution of VDR FokI genotype differed between the higher GAD-Ab titer group (LADA type 1) and lower GAD-Ab titer group (LADA type 2) ($P<0.05$) in LADA patients. The frequency of FF genotype was lower and the ff genotype higher in LADA type 1 group than that in LADA type 2 group.
Conclusion: These data suggested that the VDR gene polymorphism (FokI) might influence susceptibility to LADA and the titer of GAD-Ab in LADA in Southern Chinese.

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VDR gene polymorphisms in Chilean patients with type 1 diabetes compared to healthy controls: effect of vitamin D levels and autoantibodies profile

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Background and Aim: Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterized by the progressive destruction of insulin-producing pancreatic β-cells. The etiology of this disease is multifactorial and includes diverse environmental and genetic factors. Polymorphisms of the vitamin D receptor gene (VDR) have been associated with several autoimmune diseases, including type 1 diabetes. The aim of this study was to analyze the association between VDR polymorphisms, serological concentration of vitamin D and autoimmunity markers using Chilean type 1 diabetic cases and controls.
Subjects and Methods: The frequency of VDR polymorphisms (Taq I, Apa I, Bsm I) was obtained by PCR-RFLP from 119 cases and 193 healthy controls from Santiago, Chile. The serological concentration of 25-hydroxyvitamin D, autoantibodies GAD65 and IAA were determined by RIA. Likelihood ratio tests were performed to compare the haplotype frequency profile between cases and controls. Genotype-wise analysis of association was evaluated by means of Chi-square or fisher exact test.
Results: Genotype frequencies were not significantly different from Hardy-Weinberg expectations. We have found significant associations between two VDR polymorphisms and T1DM in our population. AA (27.7% vs 21.2%, p-value = 0.046), Bb (44.5%–36.8%, p-value = 0.037), and bb (43.7%–56.5%; p-value = 0.008) in cases versus controls respectively. A case-control difference was detected to the combined genotype (aabbTT: 15.1% in cases versus 22.8% in controls, p-value = 0.03). Haplotype analysis also yielded a significant p-value supporting association (p=0.03). Vitamin D concentration shows no difference in both groups (26.2 ± 9.7 ng/mL in cases vs. 26.6 ± 7.4 ng/mL in controls, p-value = 0.66). The presence of positive autoantibodies in patients (GAD65= 50% and IAA= 64%) was not related with and special VDR genotype
Conclusion: We have found a significant association between VDR gene polymorphisms and type 1 diabetes in a case-control study conducted in Chile (Fondecyt 1030680).
Support: Fondecyt 1030680

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Strong correlation between vitamin D receptor polymorphism and type 1 diabetes in Italian population

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Background and Aims: Type 1 diabetes reults from an immune mediated destruction of islet beta-cells. 1-25-Dihydroxivitamin D3 seems to be an immuno regulator[1-25$(OH)_2D_3$], inhibiting lymphocyte activation and affecting cytokine and immunoglobulin production. In NOD mice 1-25$(OH)_2D_3$ prevent the development of diabetes.Vitamin D exerts its genomic action through the nuclear Vitamin D receptor (VDR), which shows an extensive polymorphism. In the present study we investigated a possible association of Vitamin D receptor (VDR) gene polymorphism with type 1 diabetes in a homogeneous caucasian Italian population cohort
Materials and Methods: 246 continental italian subjects (not sardinian) affected by type I diabetes and 151 healthy subjects (controlled blood donors), matched for age and sex, have been screened for fokI and BsmI VDR polymorphisms. Genomic DNA was purified from peripheral lymphocytes using a standard proteinase K incubation followed by phenol-chloroform extraction.
Allelic specific primers were used to amplify two fragments of VDR gene including the BsmI restriction site in intron 8 and the FoKI restriction site at 5' region of the gene, respectively.
PCR products were therefore digested with specific restriction endonucleases (BsmI and FoKI) and run on a 2% ethidium bromide-agarose gel. Each subject was classified as wild type (FF or BB), heterozygous (Ff or Bb) or, finally homozygous (ff or bb).
Results: We found a significant difference about F Genotype polymorphism between diabetic patients and healthy donors (tab.1).

Tab.1

F genotype	Diabetes	%	Healthy subjects	%
f F heterozygous	112	45.5	86	57.0
f f homozygous	45	18.3	9	5.9
FF wild type	89	36.2	56	37.1
Total	246	100	151	100
P=		**0.0016**		

No significant difference was found regarding B genotype polymorphism.
A further analysis included the stratification of the patients for the combined genotypes of F and B mutations was performed.
Only a slightly significant difference was found between the two groups. An interesting observation concerns the double homozygous patients that were always diabetic. The comparison of this subgroup(14 pts) versus all the others combined was statistically significant ($p<0.002$).
Conclusion: In this study we found a strong association between VDR polymorphism and type 1 diabetes. In agreement with experimental models, we hypothesise that a decreased transcription rate with ff genotype could influence the immunoregulator activity of vitamin d and the pathogenesis of type 1 diabetes.

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Vitamin D binding protein genetic variants and type 1 diabetes

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Background and Aims: It has been shown that Vitamin D Binding Protein (VDBP) is an immunomodulator factor independently from vitamin D. Genetic variants of the VDBP in exon 11, codons 416 GAT→GAG (Asp →Glu) and 420 ACG→AAG (Thr→Lys) have been reported to be associated with type 1 diabetes (T1D). The purpose of the study is to evaluate VDBP gene polymorphisms in the Greek population and their relationship to HLA DQB1 and DQA1 alleles in patients with T1D.
Materials and Methods: VDBP gene polymorphisms 416 GAT→GAG (Asp→Glu) και 420 ACG→AAG (Thr→Lys) were evaluated by PCR-RFLP and DQB1*0201, 0301, 0302, 0602, 0603, 0604 and DQA1*02, 03, 05 alleles by PCR in 87 patients with T1D and 95 controls
Results: LysLys variant conferred higher susceptibility to T1D since it was detected in 7/87 patients (8.06%) but in none of the controls (RR 2.19, p=0.046). AspAsp/ThrThr genotype was protective for the disease since it was found in 5/95 (5.26%) controls vs 0/87 patients (0%) (RR= 0.0, p=0.05). There was no difference in frequency of LysThr, ThrThr or AspAsp, AspGlu, GluGlu genotypes between patients and controls. No correlation was found between LysLys genotype and the high risk for T1D in the Greek population HLA DQB1*0201, 0302 or 0201/0302 or DQA1
Conclusion: LysLys polymorphism in codon 420 of VDBP is detected in increased frequency among patients with T1D compared with the controls. The role of this genetic variant in the etiology of T1D needs further investigation.

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T1DBase: community website for type 1 diabetes research

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Background & aims: T1DBase (http://T1DBase.org) is a public website and database that supports the type 1 diabetes (T1D) research community. T1DBase is a collaboration between the JDRF Center for Bioinformatics at the Institute for Systems Biology (ISB) and the JDRF/ WT Diabetes and Inflammation Laboratory (DIL) at the University of Cambridge. The project is funded by the Juvenile Diabetes Research Foundation and the Wellcome Trust. Our aim is to provide T1D specific data and integrate this with general biological data to provide an overview of ongoing research. We hope to promote collaboration among researchers by allowing them to compare their own data with others and share their results. All data is open access and all software is open source.
Material & methods: T1DBase is currently focused on the molecular genetics and biology of T1D susceptibility and pathogenesis. The database contains data from human, mouse, and rat. It includes the following datasets: annotated genomes; genetically identified T1D susceptibility regions; genetic linkage and association studies (the latter in collaboration with the NIH Genetic Association Database); NOD mouse congenic strains (in collaboration with Linda Wicker); the Beta Cell Gene Expression Bank which comprises functional annotations of genes expressed in beta cells and reports expression levels of genes in beta cells under various conditions (Decio Eizirk, Laboratory for Experimental Medicine, Free University of Brussels); gene expression in a variety of tissues and organs; protein interactions from several public databases; and biological pathways from KEGG and BioCarta.
We have assembled lists of genes that are important in given pathways and are interesting for T1D research. These lists can be explored separately, together or compared with lists imported by users. Tools on the site include the GBrowse genome browser, site-wide context dependent search, Connect-the-Dots for connecting gene and other identifiers, Cytoscape for visualizing and analyzing biological networks, and the GESTALT workbench for genome annotation. All data associated with a given gene can be seen on a single page. For genes with well-defined orthologs, this page presents information from all available species. The user can control which categories of information are visible. A collaboration has been initiated with Chris Stoeckert (Computational Biology and Informatics Laboratory at the University of Pennsylvania) to functionally integrate T1DBase with EPConDB, a web resource which supports the Beta Cell Biology Consortium (BCBC). To effectively mine the T1D literature, a collaboration has been initiated with Hong Yu (Columbia University).
Results & Conclusions: Usage of T1DBase has grown ten-fold over the past year which suggests that scientists find it to be a useful site. T1DBase has been developed specifically for T1D research, but the structure and much of the general data can be applied to any human disease. We are extracting the generic aspects into a separate system, GDxBase (generic disease database), to make it easier to adapt the system to other diseases.
Support: Juvenile Diabetes Research Foundation

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Prioritizing genes under linkage peaks of genome wide scans for type 2 diabetes
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Background: Several genome wide scans have been performed to identify chromosomal regions conferring increased risk of type 2 diabetes (DM2), including the region 1q24 (from D1S498 to D1S518), 17p11.2-q22 (from D17S1303 to D17S927) and 18p11 (from D18S976 to D18S391). However, these regions are still quite large with at least 239 genes in 1q24, 289 genes in 17p11.2-q22 and 155 genes in 18p11.
Aim and Methods: To prioritize genes for further studies by identifying which of these genes show different expression in skeletal muscle between DM2 and control subjects using own and other published gene expression data sets.
Results: Out of these 683 genes from chromosome 1, 17 and 18, 425 genes were expressed in human skeletal muscle. Of them, 13 genes (*EPRS, CGI-49, FLJ10326, RAB3-GAP150, ACTN2, MYBPH, HFL3, ZNF238, MCP, DC8, LMOD1, PLXNA2,* and *TIMM17A*) on chromosome 1, 12 genes (*PRKAR1A, SFRS2, NME1, SEC14L1, HUMGT198A, AATK, ERN1, STAT3, ATP5H, MGC15396, ACTG1* and *MTMR4*) on chromosome 17 and 5 genes (*PPP4R1, DSG3, MC5R, PLEKHE1* and *PTPN2*) on chromosome 18 differed significantly between DM2 and controls. Specifically, *CGI-49* on chromosome 1 and *PRKAR1A* on chromosome 17 were highly over-expressed ($P<0.03$), while *PTPN2* on chromosome 18 was significantly down-regulated in diabetic vs. normal muscle ($P<0.02$). In another published data set 6 genes on chromosome 1, 11 genes on chromosome 17 and 1 gene on chromosome 18 under these peaks differed between DM2 and controls with no family history of DM2. One gene on chromosome 1, *TIMM17A* (a mitochondrial inner membrane translocase) was significantly down-regulated in both data-sets.
Conclusions: Bioinformatic analysis of differences in gene expression in target tissues between DM2 and control subjects may be a useful tool to prioritize genes under linkage peaks for further studies. We provide a list of such genes for skeletal muscle.
Support: Federation for Medical Bioinformatics, Karolinska Institute

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Positional candidate gene selection on chromosome 1q using bioinformatics and large scale association analysis
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Background and Aims: The increasing prevalence of type 2 diabetes reflects interaction between changing environment and genetic predisposition. Linkage of type 2 diabetes to a region of chromosome 1q21-25 has been observed in over 10 genome scans indicating that one or more T2D susceptibility genes maps to this region. In the search to identify these we are combining indirect linkage disequilibrium mapping with detailed examination of selected positional candidates.
Materials and Methods: We used multiple publicly-available sources of expression data (from both normal and T2D tissues) to grade the 481 transcripts mapping to our 31Mb region of interest, and identified four genes (*NCSTN*, *ALDH9A1*, *HDGF*, *SCAMP3*) with strong biological credentials (expression in liver, pancreas and muscle; differential expression in T2D) which had not previously been studied in detail. We typed an average of one SNP marker per 2-4 kb across each of the candidate genes in two large case-control samples (UK and French origin, total n=1499).
Results: Single-point and haplotype-based methods showed no reproducible associations with T2D for variants in *NCSTN*, *ALDH9A1* or *HDGF*. However, several variants within *SCAMP3* showed associations with T2D in both case-control sets (e. g. rs3180018, Gly3Gly, OR, for dominance of A, 1.62 (1.14–2.29) p=0.006 in French, OR 1.29 (0.99–1.70) p=0.05 in UK; rs2236863, OR, for dominance of A, 1.52 (1.07–2.15) p=0.01 and OR 1.39 (1.00–1.70) p=0.04 respectively). These SNPS have a common Minor Allele Frequency ranging from 27.5% - 31.3%. Variation in and around SCAMP3 is therefore a target for further study.
Conclusion: By integrating functional and large-scale indirect association data, we expect to accelerate the identification of T2D susceptibility genes.
Support: On behalf of the international T2D 1q consortium, funded by the NIDDK

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Chromosome 4 congenic and subcongenic BB.SHR rats as tool to identify underlying genes
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Background and Aims: Several congenic rat strains have been generated to dissect complex diseases, one of them is the congenic BB.SHR (*D4Got41-Npy-Tacr1*) rat also known as BB.LL in which a genetically defined region on chromosome 4 of diabetes-prone BB rats was replaced by that of spontaneously hypertensive rats (SHR). These rats briefly termed BB.4S develop an incomplete metabolic syndrome including obesity, hyperleptinemia, and dyslipidemia when compared with their progenitor strain BB rat. However, to identify the gene(s) the introgressed chromosomal segment must be systematically whittled down to generate recombinants and new subcongenic lines. Therefore, we generated subcongenic BB.SHR rat lines which were phenotypic analysed.
Material and Methods: BB.4S rats were crossed with BB rats, their F1 hybrids were intercrossed and genetically analysed for markers on chromosome 4. By this procedure 6 subcongenic BB.SHR rat lines – briefly termed BB.4Sa, b, c, d, e, and f – were generated. 20 males of the progenitor strain BB and BB.4S as well as 20 males of each subcongenic line were longitudinally studied from week 8 to 32 for body weight gain, glucose tolerance, serum triglycerides, total, HDL-, LDL- and VLDL-cholesterol. Serum

insulin and leptin were determined at an age of 32 weeks. All rats were killed at an age of 32 weeks and left and right inquinal adipose pads were removed to calculate the adiposity index (AI).
Results: Body weight gain (Bw) was comparable between BB.4S and their subcongenic derivatives which showed significantly higher body weight gain compared with the progenitor strain BB. Serum lipids varied more or less between BB.4S and their subcongenic derivatives, but were significantly higher in BB.4S and all subcongenics compared to their progenitor strain, BB. In contrast, serum leptin and insulin showed significant differences between BB.4S and their subcongenics. Serum leptin varied between 15.3 ± 3.5 ng/ml in BB.4S and 8.6 ± 2.0 ng/ml in BB.4Sd found in progenitor strain BB (8.0 ± 1.7 ng/ml) and 5.6 ± 1.6 ng/ml in BB.4Sc and BB.4Sf which were not significantly different from values found in the SHR donor strain (6.0 ± 1.3 ng/ml). Similar differences were also found in serum insulin. Serum leptin as well as serum insulin correlated with AI, except 2 subcongenics. But, a new phenotype was found in glucose tolerance. In contrast to BB.4S and BB.4Sf all other subcongenics showed a markedly impaired glucose tolerance (iGT) found in non-diabetic and at least 30 weeks old BB rats.
Conclusions: Based on the phenotype and genotype in BB.4S rats and their subcongenic derivatives, the most important region for Bw and serum lipids can be mapped between *D4Rat28* and *D4Rat168* (ca. 1 Mb) and for iGT between *Snca* and *D4Got72* (<1Mb) on rat chromosome 4. The syntenic regions in human are located on chromosomes 7q32 and 4q21, respectively. These synteny relationships between rat and human genes involved in facets of the metabolic syndrome makes it possible to take advantage of genetic information from studies in these rats to help in the identification of human homologoues of the genes discovered in rats.

PS 4

Genetics of type 1 diabetes: non MHC genes

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Expression profiling used for mapping susceptibility genes in a type 1 diabetes linked region on chromosome 21
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Background and Aim: In a Scandinavian Type 1 Diabetes (T1D) genome scan a region on chromosome 21 showed linkage to T1D (maximum LOD score of 2.33 (P=0.009)) in the Danish population. The identified region comprised ~ 35 cM (~20 Mb). The aim of the present study was to fine map this T1D linked region on chromosome 21, in order to be able to characterize the disease associated gene(s), explaining the observed T1D linkage.
Material and Methods: DNA from 253 Danish T1D families (1097 individuals) was used. 35 microsatellite markers were identified (covering the 35 cM region) and genotyped by PCR amplification using fluorescently labeled primers, analyzed on automated DNA sequencing equipment (ABI Prism 3100). Subsequent data analysis and multipoint linkage analysis was based on GeneMapper and GeneHunter software. SNPs were identified from dbSNP (NCBI), and genotyped by RFLP-PCR or Primer Extension reactions. T1D association was evaluated by Sib-TDT. Low Density Array cards (Applied Biosystems) for use on TaqMan 7900HT, were designed, containing gene expression assays for candidate genes. cDNA was obtained from lymphocytes from 10 newly diagnosed T1Ds and 10 controls, and from human pancreatic islets from 10 donors (unstimulated as well as stimulated with a cytokine mixture).
Results: By extended linkage analyses with additional markers, evidence for linkage was significantly increased with a peak NPL score of 3.61 (P=0.0002). The one-LOD drop support interval of 6.3 Mb was studied by linkage disequilibrium (LD) mapping with gene-based SNPs. This interval included 32 positional candidate genes and LD mapping was performed with all known SNPs (n=82) in coding regions of these genes. None of the SNPs demonstrated global association to T1D. However, analyzing subsets of data (e. g. families showing linkage), revealed T1D association of four genes (P: 0.01–0.045). The disease-related LD was also assessed by a haplotype-based association study, in which several haplotypes showed T1D association (P: 0.005–0.049). Expression profiles of all 32 genes were determined in human islets (unstimulated vs. cytokine stimulated) and in lymphocytes (controls vs. newly diagnosed T1D). A few genes markedly changed expression levels. Two of these genes also showed association in the LD analyses, and are genes involved in beta-cell function.
Conclusion: Fine mapping of a T1D linked region on chromosome 21 narrowed the linked region substantially. Evidence for the existence of one or several genes of importance in T1D in this region, was strengthened significantly by an increased NPL score of 3.61 and T1D associations of several combinations of markers, as well as markers in conditioned analyses. In addition, evaluation of expression levels of all 32 genes in lymphocytes and pancreatic islets, representing the effector cells and the target organ in T1D pathogenesis, respectively, have pin-pointed a few of the same genes with a likely functional implication in T1D, making them strong genetic and functional candidates for being responsible for the observed linkage, and supporting expression profiling as a new and valuable approach for gene mapping.

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Association of MIC-A transmembrane gene polymorphism with type 1 diabetes in the Belgian population
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Background and Aims: Different MIC-A alleles were found to be associated with Type 1 diabetes (T1D) in distinct populations. To ascertain association in the Belgian population, well-characterised antibody-positive patients were analysed for MIC-A transmembrane gene polymorphism in both an association study and a nuclear family study.
Materials and Methods: Subjects for this study were recruited by the Belgian Diabetes Registry, including 982 T1D patients, 657 control subjects

and 279 nuclear families (index patient and two parents). HLA-DQ genotypes were analysed by DQA1-DQB1 typing with allele-specific oligonucleotides, the MIC-A transmembrane trinucleotide repeat by fragment analysis.
Results: In the total study group six different MIC-A alleles were observed: A4, A5, A5.1, A6, A9 and the rare A10 allele, which was observed only once. The frequency of MIC-A5 was significantly increased in the T1D patient group (18%) compared to the control population [12%, OR=1.6 (95% CI: 1.3–1.9), $p_c < 10^{-3}$], while MIC-A9 was decreased [11% versus 18%, OR=0.7 (0.6–0.9), $p_c < 0.01$]. The UNPHASED software was used to analyse the effect of MIC-A conditioned on the HLA-DQ locus. A p-value $< 10^{-4}$ for the association of MIC-A conditional on HLA class II and $p = 0.01$ for the CETDT (conditional extended transmission disequilibrium test) were obtained, showing that MIC-A is associated with Type 1 diabetes, independently from HLA-DQ. Analysis of estimated extended HLA-DQ - MIC-A haplotypes revealed individual effects of MIC-A alleles. The most significant effect was seen for MIC-A5 on the HLA-DQA*03-DQB1*0302-MIC-A haplotype [OR=2.5 (1.4–4.2), $p < 10^{-3}$]. The same trend was observed in the family study, but was not significant. This result is reflected in the risk estimation for subjects positive for HLA-DQA*03-DQB1*0302 and positive for MIC-A5 [OR=8.0 (5.3–12.2)] or negative for MIC-A5 [OR=4.3 (3.2-5.9)], showing an additional independent effect of MIC-A5 on the HLA-DQA1*03-DQB1*0302 associated risk. However, patients stratified according to the presence or absence of the different MIC-A alleles did not differ in terms of age at onset, sex, duration of prodromal phase, HbA_{1c} expressed as the number of standard deviations above the mean of the local reference interval (SDS-HbA_{1c}), ketonuria, body mass index expressed as a standard deviation score relative to a nondiabetic control population (SDS-BMI), residual functional β-cell mass, and prevalence of the different diabetes autoantibodies.
Conclusion: MIC-A is associated with Type 1 diabetes in the Belgian population and the observed association is not due to the HLA-DQ associated risk. The strongest effect was observed for the MIC-A allele 5, which showed an additional effect on the HLA-DQA1*03-DQB1*0302 associated risk. In conclusion, MIC-A genotyping could refine genetic risk assessment in selected subjects, including first-degree relatives of T1D patients.
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Type 2 arginase gene promoter microsatellite polymorphism influences development of type 1 diabetes mellitus in Japanese
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Background and Aims: Type 2 arginase (Arg2) convert L-arginine to L-ornitine and urea, and regulate inducible nitric oxide synthase by competing the common substrate, L-arginine. In type 1 diabetes mellitus (T1DM), nitric oxide produced by immune cells infiltrating into islets plays a crucial role in beta-cell destruction. We recently found that dendritic cells from NOD mice displayed 7–10 folds reduced Arg2 mRNA expression than controls, although underlying genetic background is unclear so far. Contrary in humans, it is of interest that the Arg2 gene (14q24.1-24.3) locates closely to IDDM11 locus (14q24.3-q31), which prompted us to explore the pathogenic role of Arg2 in T1DM. In the present study, we performed a case-control study focusing on the promoter microsatellite polymorphism of the Arg 2 gene, rs3834535, reported in Japanese population.
Materials and Methods: After obtaining approval from the ethics committees, blood samples were collected from 221 T1DM subjects (103 males and 118 females) and 157 sex- and age-matched healthy subjects (80 males and 77 females). Mean age-at-onset (+ SD) was 29.0 + 16.3 years (range 8–72). All T1DM subjects were ketosis-prone and insulin-dependent since diagnosis. The promoter region of the Arg2 gene was amplified by polymerase chain reaction with primers as follows: sense: 5'-TCTCCTGTTCACTCTC-CCAGTG-3', and anti-sense: 5'-AGGGCTTCCTGTGCTACTGC-3'. Sequences of allele 1 and allele 2 of this polymorphism were t(gttt)4g and t(gttt)4tg, respectively. Compatibility to Hardy-Weinberg's law was assessed by Chi-square goodness-of-fit test. Differences of allele frequencies were statistically analysed with a Fisher's exact probability test, and P-values <0.05 considered to be significant. The strength of association was estimated by the odds ratio.
Results: Allele 2 was noted to be less common in T1DM subjects than controls (P = 0.003, Table). The significant negative association with allele 2 was observed in diabetic subjects regardless of autoantibodies (GAD and/or IA-2) positivity (Table) or onset age (data not shown). Allele distribution was not influenced by specific HLA types (data not shown). Hardy-Weinberg's equilibrium was maintained in all groups, although those homozygous for allele 2 were not identified.
Conclusion: To our knowledge, this is the first report to show that allele 2 of the Arg2 gene promoter microsatellite polymorphism serves as an important protective factor for T1DM development. This observation should be confirmed in other ethnic populations.

Table Allele frequencies of polymorphisms in the promoter region of Arg2

Allele	Subject	Allele frequency (%)	Odds ratio interval	95% Confidence	P
1	T1DM all (n=221)	93.4			
	autoantibodis positive (n=144)	92.4			
	autoantibodies negative (n=77)	95.5			
	Control (n=157)	82.7			
2	T1DM all (n=221)	6.56	0.48	0.29–0.79	0.003
	autoantibodis positive (n=144)	7.63	0.57	0.33–0.97	0.027
	autoantibodies negative (n=77)	4.54	0.33	0.15–0.72	0.003
	Control (n=157)	12.7			

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Mutation analysis of suppressor of cytokine signalling 1, a candidate gene in type 1 diabetes and insulin sensitivity
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Background and Aims: Type 1 and type 2 diabetes mellitus (T1DM and T2DM)are considered to be two distinct diseases in terms of aetiology, genetics and pathogenesis. There is, however, accumulating evidence that inflammatory mediators are of importance in the pathogenesis of both diseases. A number of natural inhibitors of cytokine signalling have recently been characterized. The suppressors of cytokine signalling (SOCS) constitute a family of proteins originally identified as negative feedback regulators of IFN- γ signalling. Emerging evidence suggests that disruption of SOCS expression or activity is associated with immune and inflammatory diseases. Mice lacking the *SOCS1* gene die within the first three weeks of life due to fatty degeneration of the liver, hematopoietic infiltration of multiple organs, lymphopenia, apoptosis in lymphoid organs and aberrant T-cell activation. SOCS1 was shown to modulate insulin signalling by targeting both IRS1 and IRS2, the two key signalling proteins in insulin action. For these reasons we consider *SOCS1*to be a candidate gene in the pathogenesis of both T1DM and insulin resistance. *SOCS1* maps to chromosome 16p13.13, a region linked to T1DM in genome scans, and consists of two exons of 104 and 1082 nucleotides. The aim of the study was to perform a complete a mutation scanning of the promoter region, the exon and the 3´UTR of the human *SOCS1* gene.
Materials and Methods: Mutation scanning was performed in 7 control subjects and 20 T1DM patients by direct sequencing.
Results: We performed a mutation scanning of approximately 3000 bp upstream of the 5´UTR region, the coding regions, intron and approximately 1000 bp of the 3´UTR region of the human *SOCS1* gene. Eight mutations were identified in the promoter region, but none in the coding regions, intron or the 3´UTR. Two of the eight mutations had allele frequencies below 1% and were not examined further whereas the remaining six -2891 C>T, -2274 T>A, -1172 T>C, -952 G>A, -926 C>T, -116 G>T was examined further. Three of the identified SNP´s were also found in the dbSNP.
In silico analyses demonstrated that four of the mutations cause changes in TF-binding sites as shown in table1.

Table 1.	Disappears	Appears
-1172: T>C	TEC1, C/EBP, Sp1	TBP, Pit-1a, YY1
-952: G>A	C/EBPalp, Sp1	AP-2alpha
-926: C>T	AP-1, CPE bind	GATA, CRE-BP1
-116: G>T	SRF, YY1, HEB, MyoD	AhR

Combination of the six polymorphisms revealed seven different haplotypes with frequencies between 0.04 and 0.37 indicating some degree of linkage disequilibrium in the region.
Conclusion: Further examinations in larger population are required in order to see if these promoter polymorphisms play a role in T1DM and insulin sensitivity. In ongoing studies of i) 250 T1DM families (1097 individuals), ii) 212 glucose tolerant first degree relatives of T2DM patients, and iii) 382 population-based young, healthy and unrelated subjects are genotyped for the six identified polymorphisms.
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The *AIRE* gene is not associated with susceptibility of type 1 diabetes
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Background and aims: Mutations in the *AIRE* gene cause a recessive Mendelian disorder autoimmune polyendocrinopathy syndrome type 1 (APS-1 or APECED). These patients develop multiorgan autoimmune diseases including type 1 diabetes (prevalence 12%). AIRE controls the central tolerance induction in the thymus. Furthermore, AIRE seems to regulate the expression level of insulin in thymus. We hypothesized that the insulin gene (*INS*) polymorphisms together with the *AIRE* variations may predispose to diabetes. The role of the *AIRE* gene was tested independently and using the *INS* genotype as a covariate in Finnish type 1 diabetes patients.
Materials and methods: A total of 368 type 1 diabetic cases and 368 matched health controls were studied. Three common single nucleotide polymorphisms (SNPs) in the *AIRE* gene having a minor allele frequency >5% were selected from the public database (dbSNP). The 23HphI polymorphism was used as a surrogate marker for the *INS* gene promoter repeat. The χ^2 test was used to compare the allele frequency differences between cases and controls.
Results: The three genotyped SNPs in the *AIRE* gene showed no evidence of association to diabetes status (p>0.3). As expected, the *INS* gene polymorphism -23Hph1 was significantly associated with susceptibility of type 1 diabetes ($p=3.0\times10^{-7}$). In the subclass of patients carrying the risk homozygote genotype of the *INS* gene the *AIRE* polymorphisms showed no association with the disease (p>0.4).
Conclusions: The *AIRE* gene does not contribute to disease susceptibility in this Finnish type 1 diabetic sample.

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1858C/T polymorphism of PTPN22 gene in the pathogenesis of type 1 diabetes
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Background and Aims: Genetic predisposition to type 1 diabetes is a result of variations in DNA sequence in different regions of genome and genes encoding proteins involved in T-cell activation (HLA class II genes, CTLA4, VNTR insulin gene) play the main role in this predisposition. The latest reports (*Nature Genetics 2004,36: 337*) suggest that another important gene associated with a higher risk of type 1 diabetes is the gene encoding protein tyrosine phosphatase N22 (PTPN22, LYP, lymphoid-specific phosphatase). The LYP phosphatase, located on the surface of the lymphocytes T, plays the fundamental role in the prevention of its spontaneous activation and reduces its reaction to antigen through dephosphorylation and inactivation of kinases associated with TCR receptor. Until now it was proved that substitution of arginine with tryptophan in 620 position of the amino acid's chain (a result of 1858C/T polymorphism) blocks LYP binding to CSK kinase's SH3 domain. As a result of this reaction "hyperactive" lymphocytes T show increased predisposition to destructive cellular immune response against its own autoantigens. The aim of our investigation was to estimate the association of the PTPN22 gene polymorphism 1858C/T with the predisposition to type 1 diabetes in Polish population.
Materials and Methods: The study was performed in the group consisting of 224 individuals with classic clinical type 1 diabetes case history (age <35 yrs.) and the control group comprising of 207 healthy controls. The presence of different variants of the investigated gene polymorphisms was estimated using the DNA sequencing method.
Results: We showed that there is a higher frequency of T allele in the group of patients with type 1 diabetes in comparison to the control group (19,7% vs.13,6% p=0,037). TT genotype occurred only in one out of 207 healthy controls, while TT homozygote was observed in 6,8% of affected subjects (p=0,005).
Conclusion: The results of our study suggest that 1858C/T PTPN22 gene polymorphism is associated with the predisposition to type 1 diabetes also in Polish population.

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Association between *HSPA1B* A(1267)G polymorphism and type 1 diabetes mellitus
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Background and Aims: The development of type 1 diabetes mellitus (T1DM) is a result of the autoimmune destruction of the pancreatic β-cells. Intracellular heat shock protein (hsp) 70 may protect β-cells against cell injury. The inducibility of *HSPA1B* (the stress-inducible form of hsp70) gene is influenced by the A(1267)G polymorphism. The carrier state of (1267)G allele is associated with lower hsp72 mRNA production. We studied the association between *HSPA1B* A(1267)G genotype and risk of T1DM.
Materials and Methods: Blood samples were taken from 375 children with T1DM. The control group consisted of 472 healthy blood donors. *HSPA1B* A(1267)G polymorphism was determined by polymerase chain reaction and restriction fragment length polymorphism method. Allele frequencies were compared using chi-square test. The study was approved by the Institutional Ethics Committee.
Results: Hardy-Weinberg criteria were fulfilled in the control population, but not in diabetic children. The distribution of *HSPA1B* (1267)GG, GA and AA genotypes was 82/229/64 in T1DM vs. 89/198/185 in the control group. The presence of the (1267)AA genotype was significantly lower in the T1DM population than in the control group.
Conclusion: This study is the first one that investigates the importance of *HSPA1B* A(1267)G polymorphism in a relatively large number of diabetic children. Our results indicate that (1267)G carrier state may be associated with the risk of T1DM. We assume that when β-cells are exposed to harmful events leading to cell destruction, (1267)G allele carriers present low hsp72 production. Low hsp72 levels may play a role in the autoimmune damage of pancreatic β-cells and contribute to the development of T1DM.
This study was supported by OTKA (Hungarian Scientific Research Foundation) Grant No T043178. Technical assistance of Ágnes Czárán and Mária Bernáth is highly appreciated.

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Differential expression and different enzyme activities of transfected antiviral 2'5'-oligoadenylate synthetase isoforms in β-TC3 cells
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Background and Aims: Type 1 diabetes is caused by a combination of multiple genetic and environmental factors that precipitate autoimmune destruction of the insulin-producing cells of the pancreas. Virus infections induce interferon-α, which through a complex signal transduction pathway induces the OAS genes encoding the key antiviral enzyme 2'5'-oligoadenylate synthetase (2'5'AS). This enzyme synthesizes oligoadenylates, which activate a latent RNase (RNaseL), which then degrades viral and cellular RNA. We recently found a highly significant association between basal 2'5'AS activity and an A/G splice acceptor site single nucleotide polymorphism (SNP) in the *OAS1* gene, indicating that 2'5'AS enzyme activity is strongly genetically controlled. Enzyme activity was highest in persons with GG genotype, intermediate in those with GA genotype and lowest in those with AA genotype. Subsequently, we confirmed the hypothesis that individuals with T1D have higher frequencies of *OAS1* GG and GA genotypes than non-diabetic control subjects. These different genotypes express different isoform profiles. The aim of the current study is to determine which isoform profile results in the lowest/highest antiviral activity and to identify the function of the new p52 isoform discovered by us.
Materials and Methods: *OAS1* splice variants corresponding to 2'5'AS isoforms were expressed in mouse βTC3 cells by transfection of various cDNA/minigene constructs. mRNA expression levels were determined by semi-quantitative PCR and isoform proteins were detected by Western

blotting. Enzyme activity of the 2'5'AS isoforms were determined by a radiometric assay.
Results: Transfection of β-TC3 cells with *OAS1* cDNA's resulted in mRNA expression of p42, p46 and p48 isoforms, and also expression of the recently discovered p52 isoform. Western blotting showed presence of isoforms p42, p46 and p52, whereas p48 was not detectable. The latter isoform was reported by others to have pro-apoptotic activity independent of 2'5'AS activity and to be detectable on Western blots only after stimulation with polyI:C and interferon-α. In our separate transfection experiments, basal enzyme activities of p52 and p48 were low compared to p42 and p46, for which activities were 8 to 20 times higher.
Conclusion: In previous studies we showed that antiviral 2'5'AS enzyme activity is controlled by an A/G splice site SNP, which is significantly associated with T1D. Results of the present study show that, in transfected cells, the p48 isoform is not detectable on Western blots and has low enzyme activity, while the p52 isoform is detectable but also has low activity. These two isoforms (p48 and p52) are produced by the A splice site allele, which is associated with reduced susceptibility to T1D, suggesting that both isoforms could be protective in relation to T1D.
The studies are supported by grants from JDRF and NIH

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Phenotypic associations of KIR 6.2 mutations

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***PPARG* P12A, Kir6.2/*KCNJ11* E23K and early growth in the Northern Finland Birth Cohort of 1966**
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Background and Aims: The *PPARG* P12A and Kir6.2/*KCNJ11* E23K variants have been repeatedly associated with an increased susceptibility to type 2 diabetes. One possible explanation of observed relationships between diabetes in adulthood and poor early growth may be shared genetic determinants of both. Using a large Finnish birth cohort, our aim was to test this hypothesis by establishing whether or not these known diabetes-susceptibility variants were also associated with early growth phenotypes, such as birth weight, birth length, ponderal index, placental weight and head circumference at 1year.
Materials and Methods: 5332 and 5180 individuals from the Northern Finland Birth Cohort of 1966 were successfully genotyped for the P12A and E23K variants respectively using Taqman Assays on Demand. Linear regression was used to investigate possible associations. Recessive, additive and dominant models were considered, with stratification by gender and adjustment for other possible confounding and/or contributory factors (e. g. maternal smoking, gestational age).
Results: *PPARG* P12A results: No significant associations were seen for birth weight, birth length, ponderal index, placental weight or head circumference at 1year, under any model or stratification considered. Kir6.2/*KCNJ11* E23K results*:* For the majority of analyses there were no significant associations between E32K genotype and the early growth phenotypes considered. In gender-specific analyses, birth weight showed a nominally-significant relationship in females (30g higher (95%CI 5–56g) for each K allele; p= 0.017, fully adjusted additive model). This association was also detected in the model recessive for the K allele (fully adjusted p= 0.041). However, in the absence of a prior hypothesis for gender interaction, this result was considered likely to be the result of multiple testing.
Conclusion: In this study, one of the largest to date of the *PPARG* P12A and Kir6.2/*KCNJ11* E23K variants and early growth variables, we have failed to find any consistent associations. We are unable to substantiate the hypothesis that the pleiotropic effects of diabetes-susceptibility variants on early growth, contribute to observed epidemiological associations.

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Kir6.2 mutations search in patients with permanent neonatal diabetes from a Polish population
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Background and Aims: Activating mutations in the *KCJN11* gene encoding Kir6.2, the ATP-sensitive potassium-channel subunit have been recently described in patients with permanent neonatal diabetes (PND). The aim of this study was to examine the contribution of *KCJN11* mutations to PND in patients from Poland.
Methods: We sequenced the *KCJN11* gene in 7 insulin treated, diabetic patients diagnosed before 6 months (range 1–26 weeks).
Results: We identified three patients with de novo heterozygous missense mutations. Two males, (Pol1 and Pol2) carried the previously described R201H mutation and one female (Pol3) diagnosed at 26 weeks was a carrier of novel mutation R50Q. All three subjects with Kir6.2 mutations presented

with severe hyperglycemia at the diagnosis (30–50 mmol/l). There was evidence of clinical heterogeneity: the age of diagnosis was 2, 3 and 26 weeks in Pol1, Pol2, Poll3 respectively; all were born at term with birth weights of 2450, 2700 & 3000g and insulin requirements varied 0.25, 0.68, 0.1 U/kg. All three patients were successfully transferred to sulphonylurea therapy. In each case we used controlled-release glipizide GITS (gastrointestinal therapeutic system). For Pol1 and Pol3 5 mg of glipizide was used, while Pol2 daily requirements was 30 mg.
Conclusion: We summarize that Kir6.2 mutations are a common cause of PND in European Caucasians and provide the evidence of clinical heterogeneity between mutation carriers. The clinical results are consistent with the novel mutation R50Q being less severe than R201H although variation between patients with R201H suggest there may be other genetic or environmental moderators of phenotype. We report the first successful initial transfer of three patients from insulin to a sustained release sulphonulyrea.

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Apparently insulin-dependent patients with neonatal diabetes due to mutations in Kir6.2 may be managed on sulfonylurea with sustained metabolic control rather than insulin injections

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Background and Aims: Recently, we have recently shown that sulfonylurea tablets can replace insulin injections in permanent neonatal diabetes due to mutations in the ATP-potassium-channel subunit Kir6.2. We wanted to monitor the long-term effect of this treatment.
Materials and Methods: Thirteen patients with Kir6.2-PNDM were invited to participate in a study investigating the effect of sulfonylurea with endpoints insulin requirement and HbA1c. Here, we provide data for eight subjects that have so far completed the treatment protocol.
Results: Five subjects had the mutation R201C, the others had either F35V, V59M or F333I. Clinical characteristics were (median, range in parenthesis): birth weight, 3080 g (2410–3260); age at diagnosis, 26 weeks (20–55); serum glucose at presentation, 7 mmol/L (1–12). Before entering the treatment study, the subjects had an insulin requirement of 0.57 U/kg/day (0.22–0.70); HbA1c was 7.7% (7.1-8.9); C-peptide concentrations and paired serum glucose were 0.19 nmol/L (0.01–0.50) and 10.3 mmol/L (7.3–22.6), respectively. All subjects who have completed the protocol responded well to sulfonylurea tablets. Insulin injections were discontinued. None had notable side effects. Presently, the subjects have been treated for nine months (median, range 6–16). Recent HbA1c was 5.4% (median, range 5.4–6.9), which is lower than prior to treatment (p=0.0005). One subject has been off insulin for 16 months. Several HbA1c measurements two years before switching to insulin were mean 8.1% 0.5, and after insulin was discontinued mean 6.5% 0.8 (p=0.0004).
Conclusion: Apparently insulin-dependent patients with mutations in Kir6.2 may be managed on an oral sulfonylurea with sustained metabolic control rather than insulin injections.

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A *KCNJ11* mutation in the ATP-binding site of the K_{ATP} channel causes neonatal diabetes with epilepsy

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Background and Aims: ATP-sensitive K^+ (K_{ATP}) channels link cell metabolism to electrical excitability of the plasma membrane. They are widely expressed in endocrine cells, heart, skeletal muscle, peripheral nerve and brain and play diverse roles in insulin secretion, neuronal excitability, cardiac stress and the response to cardiac and cerebral ischemia. They comprise pore-forming Kir6.2 and regulatory sulphonylurea receptor (SUR) subunits. Metabolic regulation is mediated by changes in intracellular adenine nucleotides. ATP binding to Kir6.2 inhibits K_{ATP} channel activity, and interaction of Mg-nucleotides with SUR increases channel activity: thus in the presence of MgATP, channel activity reflects a balance between these inhibitory and stimulatory actions.
Heterozygous (*het*) mutations in Kir6.2 (*KCNJ11*) cause permanent neonatal diabetes either alone (PNDM) or in association with developmental delay, muscle weakness, and epilepsy (DEND syndrome). All mutations studied to date act by reducing the ATP sensitivity of the K_{ATP} channel. In general, mutations that cause PNDM impair ATP binding and/or transduction of ATP binding into pore closure, whereas mutations that cause DEND syndrome act indirectly, by stabilizing the open state of the channel which thereby decreases ATP block. We now report a mutation, R50P, in the putative ATP-binding site of Kir6.2 that severely impairs ATP sensitivity and results in DEND syndrome.
Materials and Methods: K_{ATP} currents were recorded from *Xenopus laevis* oocytes injected with wild-type (*wt*) or mutant Kir6.2 and SUR1 mRNAs. To simulate the *het* state, we used a 1:1 mixture of *wt* and mutant Kir6.2 mRNAs. Currents were recorded by patch-clamping inside-out membrane patches. The pipette solution contained (mM): 140 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$, 10 HEPES (pH 7.4). The Mg^{2+}-free internal (bath) solution contained (mM): 107 KCl, 1 K_2SO_4, 10 EGTA, 10 HEPES (pH 7.2). The Mg^{2+}-containing internal solution consisted of Mg^{2+}-free solution plus 2 mM $MgCl_2$ and MgATP (instead of ATP).
Results: The R50P mutation caused a marked decrease in K_{ATP} channel sensitivity to ATP. In Mg^{2+}-free solution, IC_{50} for ATP inhibition were 4.9 ± 0.1 µM (n=5) for *wt* and 106 ± 9 µM (n=6) for hetR50P channels. Homozygous R50P channels were not blocked even by 5 mM ATP. In the presence of Mg^{2+}, $IC_{50} = 26.9 \pm 2.4$ µM (n=5) for *wt* and 50 ± 17 µM (n=6) for *het*R50P channels. Homozygous R50P channels were *activated* ~3-fold, reflecting the stimulatory action of MgATP at SUR1. Importantly, 36% of *het*R50P current was not blocked even at saturating [MgATP], as reported for other DEND syndrome mutations (eg. I296L). In contrast to other DEND mutations, the single channel kinetics were unaffected, indicating the R50P mutation affects only ATP binding/transduction. Furthermore, unlike other DEND syndrome mutations, whole-cell K_{ATP} currents were only slightly less sensitive to tolbutamide block than *wt*.
Conclusion: These data demonstrate that severe functional mutations in the ATP-binding site, which do not affect channel gating, can cause DEND syndrome. The fact that the single-channel kinetics are unaffected probably explains the near-normal tolbutamide sensitivity and suggests that some extra-pancreatic symptoms may respond to sulphonylurea therapy.

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Insulin independence with sulphonylurea treatment in a series of patients with permanent neonatal diabetes due to heterozygous activating mutations in the KCNJ11 (Kir6.2) gene

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Background and Aims: The finding that activating mutations in *KCNJ11* (encoding the Kir6.2 subunit of the K_{ATP} channel) are a common cause for permanent neonatal diabetes raised the possibility of sulphonylurea treatment in these "insulin dependent" patients. We aimed to investigate the response to sulphonylurea treatment in a large heterogeneous series of patients with Kir6.2 mutations.
Materials and Methods: A sulphonylurea was introduced gradually and insulin dose reduced when possible until insulin could be stopped or until at least the equivalent of 1 mg/kg/day glibenclamide was achieved. HbA1c (n=8) and insulin increment to intravenous glucose (0.3g/kg) (n=5) pre and post transfer to sulphonylureas were compared by Wilcoxon-Ranks test.
Results: Of 16 patients trialled with sulphonylureas, 14 were able to stop insulin treatment (mutations R201H (7), R201C (2), R201L, V59M (2), K170T, G53N). Median age at transfer was 5 years requiring the equivalent of glibenclamide 0.65 mg/kg/day (normal max adult dose 0.25 mg/kg/day). Even with the same mutation (R201H) the range of glibenclamide dose was 0.05 to 1.3 mg/kg/day. HbA1c improved in all patients with a median pre-sulphonylurea HbA1c of 7.5%, and post transfer HbA1c of 6.5% (p=0.012). Response to sulphonylureas was maintained at medium term follow up (median 6 months). The median insulin increment to i.v. glucose following transfer to sulphonylurea was small (20pmol/l) and not significantly increased from the pre-sulphonylurea increment (3pmol/l, p=0.14). One patient (G53R) age 43 years had reduced insulin requirements by 66% with 1.2 mg/kg/day glibenclamide,

and one 4 year old with a V59M mutation had no reduction in insulin requirements at 1 mg/kg/day glibenclamide.
Conclusions: Transfer from insulin to sulphonylureas with improvement in glycaemic control is successful in patients with a variety of Kir6.2 mutations. Sulphonylurea requirements are, on average, 2.5 times higher than in type 2 diabetes. The dramatic improvement in HbA1c is not reflected by an increased insulin secretory response to intravenous glucose following sulphonylurea treatment. This suggests that sulphonylurea treatment enables non-K_{ATP} mechanisms to influence insulin secretion, rather than enhancing the direct glucose-dependent ATP mediated pathway.
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Congenital, transient or focal hyperinsulinism, adult hypoglycaemia, gestational diabetes and insulin resistance caused by heterozygous inhibiting mutations in KCNJ11
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The K_{ATP} -channel of the pancreatic β-cell controls insulin release. The channel consists of four Kir6.2 subunits and four SUR1 subunits. Congenital Hyperinsulinism (CHI) can be caused by mutations in the KCNJ11 gene encoding Kir6.2. 115 CHI patients was analysed for mutations in SUR1 and Kir6.2, using DHPLC and sequencing. We found two children (1.7%) with KCNJ11 mutations. Patient 1 had a novel, highly conserved maternal mutation, R177K, and a mild, transient CHI. Her mother had gestational diabetes and intermittent hypoglycemia. Patient 2 had a novel, highly conserved paternal mutation, E282K and focal CHI. His heterozygous family members had intermittent or no hypoglycemic symptoms and a borderline low, or normal, HbA1c. Their fasting blood glucoses were normal, but 2-h OGTT blood glucoses were low or normal with elevated fasting and 2-h OGTT plasma proinsulin and plasma C-peptide levels suggesting insulin resistance.
In recombinant in vitro homozygous model cell systems, the mutant channels produced no functional current. The R177K channels displayed correct membrane trafficking, whereas the K282 channels were retained in the endoplasmic reticulum. In similar heterozygous models, channel function was in-completely recovered, suggesting a mild inhibiting effect of the mutations.
Thus, heterozygous inactivating KCNJ11 mutations may cause mild, transient CHI, adult intermittent hypoglycemia, gestational diabetes or insulin resistance, and contribute to focal CHI.

PS 6
Epidemiology of type 1 diabetes II

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Children who develop type 1 diabetes early in life have low levels of carnitine and amino acids at birth: does this finding shed light on the etiopathogenesis of the disease?
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Background and Aims: at the time of diagnosis of type 1 diabetes (T1D), patients showed an altered carnitine profile, but no investigations have analysed this pattern in individuals before the onset of the disease. We have hypothesized that the carnitine impairment might be evident before the clinical appearance of T1D, possibly from birth. Thus, we have carried out a retrospective case-control study, aimed at investigating circulating carnitine and amino acids in the first days of life, and their relationship with the future onset of T1D.
Materials and Methods: diabetic patients were identified within the Unit of Diabetology at the Bambino Gesù Paediatric Hospital in Rome. Controls were selected from 1,650 children, who were HLA typed at birth for determining their genetic susceptibility to T1D. As criteria of inclusion, children had to be born in the Lazio region between January 2000 and December 2002 and T1D had to be developed by the age of 4 years. These criteria were adopted in order to: i) have the possibility to retrieve dry blood spots, ii) reduce the time elapsing from birth and assays, and iii) identify controls matched for age, sex and HLA genetic susceptibility to T1D. Overall, 11 diabetic children fulfilled these criteria and 44 matched controls were included. Information regarding birth weight, gestational age and feeding received soon after birth, i.e. breast or formula milk, was recorded. Dry blood spots were retrieved from the 2 centers in charge for neonatal screenings in Lazio. All spots were collected within the first 3 days after birth and stored at room temperature up to the analysis. In each blood spot, concentrations of total- (TC) and free-carnitine (FC), acyl-carnitines (AC) and of 13 amino acids (essential: Leu/Ile, Met, Phe and Val; non essential: Ala, Asp, Arg, Cit, Gly, Glu/Gln, Orn, Pro and Tyr) were determined by tandem mass spectrometry (API Sciex 365, PE Sciex Instrument, Concord, ON, Canada).
Results: among the 11 T1D children, 4 were at high HLA genetic risk, 6 were at moderate risk and 1 was at low risk. The median age at the T1D onset was 2.7 years, ranging from 1.1 to 3.8 years. No differences between diabetics and controls were seen in reference to the gestational age, birth weight and feeding (P=0.438, P=0.408 and P=0.522, respectively). The time elapsing from the collection of dry blood spots and the assays was comparable in the two groups (P=0.191). In contrast, circulating TC, FC and AC were significantly lower in T1D patients compared to controls (P=0.004, P=0.009 and P=0.009, respectively). Furthermore, total amino acid concentrations, expressed as the algebraic sum of all amino acids tested, were also significantly lower in T1D patients than in controls (P=0.003), even when clustered in the essential and non essential subgroups (P=0.003).
Conclusion: this is the first study demonstrating that children who develop T1D early in life showed reduced circulating carnitine and amino acid levels soon after birth. Their evaluation in the early neonatal period could represent an additional tool in the prediction of T1D and offer new strategies for possibly preventing the disease as early as from birth.
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Timing of cereal introduction in the infant diet and risk of islet autoimmunity, celiac disease autoimmunity and wheat allergy: a clue to a common etiology?
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Background and Aims: Infant diet exposures have long been of interest to those investigating the etiology of type 1 diabetes mellitus (T1DM), celiac disease and food allergy. However, the studies to date have been inconsistent.
Materials and Methods: In the Diabetes Autoimmunity Study in the Young (DAISY), we have enrolled over 1500 children at increased risk for T1DM or CD, as defined by either HLA genotype or having a first-degree relative with diabetes, and followed them from birth for a mean of over 4 years for

the development of autoantibodies related to diabetes and celiac disease. Infant diet data were collected prospectively via parental interview. Islet autoimmunity (IA) (n=34 cases out of 1183 children) is defined as presence of one of three islet autoantibodies on two or more consecutive visits and still positive or diabetic on last follow-up. Celiac disease autoimmunity (CDA) (n=51 cases out of 1560 children) is defined as positive tissue transglutaminase antibodies (tTG) on two or more consecutive visits, or once plus a positive small bowel biopsy for CD. Cases of wheat allergy (n=15) were determined by parental report of the allergy; children were excluded from this definition if they were also tTG positive to avoid misclassification with CD. We are currently testing case plasma for IgE to gluten. Multivariate survival analysis using the Weibull distribution was used to examine risk factors for the development of IA and CDA. Logistic regression was used to examine risk factors for the presence of wheat allergy. The IA model was adjusted for HLA, breast-feeding, family history of T1DM, ethnicity, maternal age. The CDA model was adjusted for HLA, and the wheat allergy model was adjusted for HLA and family history of allergy.
Results: Both early (0–3 months) and late (7+ months) introduction of any type of cereal into the infant diet was associated with increased risk of IA (JAMA 2003;290:1713–20). Similar associations with timing of introduction were seen for CDA and wheat allergy, but were specific to introduction of gluten-containing cereals. (see Table)
Conclusion: Timing of cereal exposure may play an important role in the risk of T1DM, CD and wheat allergy, suggesting a common etiology in these seemingly different diseases.
Timing of introduction of cereals and risk of three disease outcomes

	Disease Outcome Islet Autoimmunity	Celiac Disease Autoimmunity	Wheat Allergy
Age Introduced	HR (95% CI)*	HR (95% CI)#	OR (95% CI)#
0–3 months	4.3 (2.0–9.3)	5.2 (1.4–18.6)	3.3 (0.3–32.6)
4–6 months	1.0 (ref)	1.0 (ref)	1.0 (ref)
7+ months	5.4 (2.1–13.8)	1.9 (0.97–3.6)	5.1 (1.4–18.6)

* For exposure to any cereal
\# For exposure to gluten-containing cereals

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Menopause in type 1 diabetic women
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Background and aims: Currently there are about 1.2 million females with type 1 diabetes in Europe. Epidemiological data on menopause in type 1 diabetic females are very few. Menopause is one of the most important periods of female life with regard to health issues. Premature menopause is considered a risk factor for cardiovascular disease and increased cardiovascular and total mortality. Since diabetes in itself increases the cardiovascular risks, a premature menopause among diabetics would mean an even larger risk clustering among type 1 diabetic women. The objective of this research is to study the physiologic phenomenon of menopause in a large population-based representative group of Finnish women with childhood-onset type 1 diabetes diagnosed in 1965–1979.
Materials and methods: The population-based cohort that is subjected to study was initially identified for the study of the mortality of type 1 diabetic patients – the Diabetes Epidemiology Research International (DERI) Group's mortality study, the subjects of which were diagnosed with diabetes at 17 years of age or under during 1965 and 1979; placed on insulin at diagnosis; and residing in Finland. In the registry, 5,162 cases were identified during 1965–1985 with an ascertainment rate approaching 100 percent. Of those approximately half were women. According to the latest follow-up of the DERI cohort, the number of women with type 1 diabetes who were potentially in the perimenopausal period (age range 40–55 years) at the start of this study (2002) is 1004. Of these 1004 women, 980 could be contacted and were sent a detailed questionnaire on their gynecological and reproductive history, diabetes-related facts, life-style factors and other diseases. 561 questionnaires were returned, giving a response rate of 57%.
Results: Of the 561 respondents, 61 (11%) had undergone hysterectomy and 41 (7%) used a hormonal intrauterine device causing amenorrhea. These women were excluded from the analyses. There were 51 women who had experienced non-surgical menopause. Of these, 11 had started using hormonal replacement therapy (HRT) while still menstruating and were thus not able to report their menopausal age. In this small group, HRT was started at the age of 44.5 +/– 7.1 years (mean +/– SD). Those who could report their age at the cessation of menstruation (n=40) had a menopausal age of 46.5 +/– 3.7 years (mean +/– SD). Among those women still premenopausal, there were 20 women of 50–54 years of age, 157 women in the age group 45–49 and 215 in the age group 40–44 years. Those who had experienced menopause by the time of the study did not differ from the premenopausal ones in regards of the number of deliveries, spontaneous abortions, extrauterine pregnancies or induced abortions. The total number of pregnancies was slightly smaller among those having experienced menopause, but the difference was not statistically significant.
Conclusions: The mean menopausal age in Finland is 51 years. The results of this study suggest a somewhat earlier menopausal age among type 1 diabetics. A possible relationship between the degree of diabetic complications and earlier menopausal age remains to be analyzed in this cohort of diabetics.
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Mortality and causes of death in childhood onset type 1 diabetes in Norway: a population-based study
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Background and Aims: In spite of significant reduction in mortality after the introduction of insulin therapy, type 1 diabetic patients still experience a markedly reduced lifespan compared with non diabetic individuals. The study aimed to examine the mortality rates, excess mortality and causes of death in childhood onset type 1 diabetes in Norway.
Materials and Methods: All individuals in Norway with childhood onset type 1 diabetes (0–14 years) from 1973 through 1982 were included, 1906 subjects. Mortality was recorded from diabetes onset and until 31st December 2002, representing 46 147 person-years of risk. By end of follow-up the highest possible attained age was 44 years and maximum diabetes duration 30 years. The mortality status was determined by matching the unique personal identification number assigned to each resident of Norway to the vital statistical data. Excess mortality, compared with the background population, was assessed in term of standardized mortality ratios (SMR) for the total cohort and for both genders. A review of death certificates, autopsy protocols and hospital records revealed the cause of death.
Results: A total of 103 deaths were identified. Annual mortality rate was 2.2/1000. Mean age at death was 26.1 ± 7.8 (mean±SD) years (range 0.8–40.2). The mean duration of follow-up was 24.2 ± 3.9 years (0.0–30.0). SMR in the diabetes cohort was 3.98 (95% CI: 3.22–4.75), and there was no significant difference between men and women (3.92 vs. 4.02). Subjects with age 10–14 years at diabetes onset had significant increased mortality rate compared to onset below 10 years of age (RR=1.7, 95% CI: 1.15– 2.51). Acute metabolic complications of diabetes was the greatest single cause of death under the age of 30 years. Cardiovascular disease (CVD) was responsible for the greatest proportion of deaths from the age of 30 years onwards. At all ages deaths certified to CVD exceeded those certified to renal disease. Violent death, including fatal accidents, suicide, violence and intoxication, accounted for 28% of the deaths in the total cohort, mainly in men 35% compared to 11% in women.
Conclusion: Childhood onset type 1 diabetes still carries an increased mortality risk when compared with the non diabetic population. The excess mortality was similar for males and females. To reduce these deaths attention must be directed to prevention of acute metabolic complications, identification of psychiatric vulnerability and early detection and treatment of cardiovascular disease and associated risk factors.
This study was supported by grants from the Norwegian Diabetes Association and Aker Diabetes Research Foundation.

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Insulin secretion at diagnosis of type 1 diabetes is associated with both age of onset and HLA class II
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Background and Aims: We investigated whether residual insulin secretion and metabolic derangement at diagnosis of Type 1 diabetes (T1DM) are

influenced by Human Leukocyte Antigens (HLA) class II genes.Although previous studies demonstrated that HLA susceptible genotypes are important determinants of earlier disease onset, and that young age at diagnosis is associated with lower C-peptide levels, the relationship between HLA class II and beta-cells function did not lead to conclusive reports. We investigated whether residual insulin secretion and metabolic derangement at diagnosis of T1DM are influenced by HLA.
Materials and Methods: 871 T1DM consecutive Caucasian patients were typed for HLA class II genes. In 300 of these patients, glycated haemoglobin, insulin requirement, baseline C-peptide and Body Mass Index (BMI) Z-score were measured at clinical diagnosis. The effect of the HLA genotypes on the quantitative variables was investigated using multiple linear regression. The beta coefficient regression of the age at onset and HLA genotypes, were standardized to compare their specific importance for C-peptide levels.
Results: The HLA genotypes were divided in high, moderate and low-risk categories. The frequency of high risk genotype, DRB1*03-DQB1*0201/DRB1*04-DQB1*0302, decreased with increasing age of onset ($p< 0.0001$, χ^2 linear trend). The presence of the high risk genotype was independently associated with lower C-peptide levels at diagnosis ($p= 0.002$). In the regression analysis of C-peptide levels, the standardized beta coefficient for age of onset and high risk compared to low risk genotypes showed similar results (0.27 and 0.24 respectively).
There was a positive association between age of onset and C-peptide ($p<0.0001$) and a negative association between age of onset and insulin requirement ($p< 0.0001$).
Conclusion: The degree of beta-cell destruction at diagnosis of T1DM is independently associated with both age of onset and HLA genotypes, the two variables exert a similar quantitative effect on residual beta-cell function at diagnosis.

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Five years follow-up study of Japanese patients of latent autoimmune diabetes in adults (LADA) in Ehime Study

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Background and Aims: We previously screened autoantibody against GAD (GADA) in adult-onset Japanese diabetic patients, and reported differences in the contribution of HLA class II haplotypes to susceptibility to type 1 diabetes (T1DM) in Ehime Study. To clarify the natural course of LADA patients and factors which are involved in the deterioration of β-cell function, we followed up the LADA patients in Ehime Study, and analyzed their clinical and immunogenetic characteristics.
Materials and Methods: GADA was screened in hospital-based samples from 4,980 adult-onset (> 20 years) diabetic patients in the Ehime area of Shikoku Island in Japan as a part of the Ehime Study during 1998 and 1999. In patients positive for GADA without insulin treatment at the registration, their insulin secretion and clinical characteristics were analyzed after five years. Insulin secretion was assessed by postprandial C-peptide levels. Patients who progressed to insulin-deficient state (ID) (below 0.33 nmol/l of serum C-peptide at 2 h postprandial) were compared with those with non-insulin-deficient state (NID). One hundred ninety subjects with a normal glucose tolerance served as controls.
Results: Of the 4,980 adult-onset diabetic patients, GADA was detected in 188 patients (3.8%). Of the 188 GADA patients, 57 were not treated with insulin. Among these 57 patients, 40 patients whose clinical information was available after 5 years follow-up were analyzed. Five patients progressed to ID and 35 patients were NID. Fasting and postprandial CPR levels at registration were lower in ID group (ID 0.88 ± 0.42 and 2.13 ± 1.08nmol/l vs. NID 2.50 ± 0.37 and 5.44 ± 2.80nmol/l, $p<0.05$). Titer of GADA and positivity of IA-2 and thyroid-related autoantibodies at registration were higher in ID group than those of NID group ($p<0.05$). On class II HLA, frequencies of T1DM susceptible HLA haplotypes in Japanese, *DRB1*0405-DQB1*0401*, *DRB1*0802-DQB1*0302* and *DRB1*0901-DQB1*0303* were not different between ID and NID groups (50% vs. 39.9%), but tended to be higher than those of controls (28.9%, NS). T1DM-resistant HLA haplotypes (*DR2*) were not found in ID group, although 8 patients in NID group (23%) had *DR2*. When compared to 63 patients of acute onset T1DM in Ehime Study (AID), homozygotes of neutral haplotypes to T1DM were frequent in ID group (ID 40% vs. AID 3.2%, $p<0.05$).
Conclusion: During the 5 years follow-up, 12.5% of LADA patients developed ID. Multiple positivity of autoantibodies and lower CPR levels at registration may be risk factors for ID. Frequencies of HLA TIDM susceptible haplotypes were not different between ID and NID groups, but *DR2* haplotypes were protective to ID. Further work for contribution of neutral haplotypes to T1DM will be needed to clarify risk factors involved in the development of ID.

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Monogenic forms of diabetes

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The role for complement C5 and C8, apoM and transthyretin as biomarkers for MODY 1 and 3

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Background and Aims: So far, six different forms of MODY have been described. Genetic testing is needed for the diagnosis of MODY but genetic testing is expensive and time consuming. Thus, there is need for additional approaches for identifying different types of MODY. Biomarkers for MODY could be of great value in this respect and also provide knowledge about the nature of different forms of MODY. Complement 5 (C5) and 8 (C8), apolipoproteinM (apoM) and transthyretin (TTR) have been suggested to be regulated by transcription factors HNF-1α and HNF-4α. Circulating C5 and C8 have been shown to be decreased in an HNF-1α KO-mouse. ApoM was recently suggested to be decreased in an HNF-1α KO-mouse and in patients with MODY 3.The aim of our work was to study the levels of the liver derived proteins C5 and C8, apoM and TTR in patients with MODY 1 (M1), MODY 3 (M3) and type 2 diabetes (T2D) to examine their potential roles as biomarkers for MODY.
Materials and Methods: Blood samples were collected from patients with identified mutations in either the HNF-1α or HNF-4α gene from our clinic, for further analysis of C5, C8, apoM and TTR. All samples were freshly frozen and stored at −70 °C prior to analyzes. BMI-matched (BMI ≤30), GAD negative T2D patients with diabetes onset ≥40 years were used as controls and BMI matched healthy individuals as an additional control group.
Results: A marked decrease in C5 and C8 was seen in both M1 and M3 patients compared to T2D patients (M1: C5:63%, C8:48% of control, $P<0.01$ and M3: C5:72%, C8:69% of control, $p<0.01$, $p<0.05$). The most marked effect was seen for C8 in M1 subjects which was decreased also compared to healthy controls. The expression of C5 appeared to be twice as high in T2D patients compared to non diabetic subjects. The concentration of C5 and apoM showed a positive correlation to the metabolic control, whereas no such correlation was seen for C8 or TTR. The levels of C5, C8, apoM or TTR did not show any correlation to BMI, W-to-H or body fat (%). M1 patients showed a 26% reduction in circulating TTR compared to T2D patients. The TTR concentration did not differ between subjects with M3, T2D and non diabetic subjects. ApoM was significantly lower in M1 patients than in healthy controls and tended to be so compared to T2D patients, whereas no alteration was seen in M3 subjects (M1: 0.70, M3:0.96, T2D: 0.94, control: 0.99, $p<0.05$).
Conclusion: Our data suggest that complement 5 and 8 may have a role as biomarkers for MODY 1 and 3 and that apoM and transthyretin could add information particularly on MODY 1s. C8 seems to be the most robust biomarker which is markedly decreased in MODY patients compared to both T2D and controls and uninfluenced by metabolic control and BMI.

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Glucokinase and hepatocyte nuclear factor 1 alpha gene mutations in Greek families with MODY. Higher incidence of glucokinase gene mutations (MODY2) in the Greek population

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Background and Aims: Maturity Onset Diabetes of the Young (MODY) is a monogenic form of diabetes mellitus characterized by autosomal dominant inheritance, early age of onset (typically but not exclusively before the age of 25 years) and deficient pancreatic beta cell function. To date, mutations in six genes have been associated with different subtypes of MODY (MODY 1 to 6), making it a clinically and genetically heterogeneous group of disorders. The most common of these subtypes, in various European populations, are MODY 2 (Glucokinase gene) and MODY 3 (HNF1α).
Materials and Methods: We screened 200 members from 60 families of Greek origin with clinical features indicative of MODY for the presence of mutations in the Glucokinase (GCK, MODY2) and Hepatocyte Nuclear Factor 1α (HNF1α, MODY3) genes, employing the method of Denaturing Gradient Gel Electrophoresis (DGGE). Genomic DNA was isolated from peripheral blood lymphocytes. Exons 2 to 9 of the GCK and 1 to 10 of the HNF1α genes were PCR-amplified using primers appropriately designed for DGGE analysis. The samples with altered band patterns after DGGE, were re-amplified and directly sequenced in order to identify the mutation.
Results: We identified 16 mutations in the GCK gene in 21 families (35%). Ten of these are novel mutations: Gln24stop, Tyr61stop, Ser263Thr, Gly249Ser, Val253Ala, Glu265Lys, Glu272Gly, Glu300Lys, Leu352Pro, and the splice site mutation IV5+1G>C. In thirteen families, the mutations were of paternal origin, in seven families of maternal origin and one was a *de novo* mutation. Three of our patients (5%) had HNF1α gene mutations that were reported previously.
In all patients, low insulin values were detected. The patients with GCK mutations had mild hyperglycemia (<130 mg/dl) managed by diet with no deterioration over many years of follow-up. In contrast, patients with HNF1α mutations had higher glucose levels (>200 mg/dl).
Conclusion: The frequency of GCK mutations in Greek patients with MODY is much higher than that of HNF1α, as is the case in the French, Italian and Spanish, but not British population. The identification of the genetic defect in patients with clinically suspected MODY is important to determine prognosis and for genetic counseling.

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Renal agenesis and genital malformation may be linked to mutations in the hepatocyte nuclear factor-1alpha (MODY3) gene

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Background: Maturity-onset diabetes of the young (MODY) is an autosomal dominant form of diabetes characterised by beta-cell defect and early age of diagnosis. MODY is sometimes accompanied by extrapancreatic features. The most frequent MODY3 subtype is caused by mutations in the hepatocyte nuclear factor (HNF)-1alpha.
Aim: To determine the frequency of diabetes due to mutations in the HNF-1alpha gene in MODY families from the Polish Nationwide Registry and to provide its clinical characteristics.
Materials and Methods: We identified 25 families with early onset, autosomal dominant form of diabetes that meet the criteria of MODY. The 10 exons and promoter region of the gene were screened for sequence differences by direct sequencing of DNA from the probands of these families.
Results: So far, during a screening of probands from MODY families of Polish origin we identified 6 mutations. They segregated with diabetes in families where they were identified. Four of them were missense mutations, two previously identified (Arg131Gln and Arg271Trp), and two newly discovered (Ser249Pro and Asn257Thr). Two others included the previously published frameshift Pro379fsdelCT mutation in exon 6 and the cryptic splice acceptor site mutation IVS7nt-6G>A. The latter mutation was earlier reported to result in the skipping of exon 7 and premature termination codon. Interestingly, the proband from the family with the IVS7nt-6G>A mutation was diagnosed with kidney agenesis and genital malformation (a bicornuate uterus). In addition, one patient from a family with the Arg271Trp mutation that was diagnosed with diabetes at the age of 15 years showed the phenotype of a single functioning kidney. Since there were several earlier reports on mutations in the hepatocyte nuclear factor (HNF)-1beta gene resulting in disorders of renal and genital development and early-onset diabetes (MODY5), we performed a screening for mutation in this gene in the IVS7nt-6G>A mutation carrier that produced negative results. The search for HNF-1beta sequence differences in the patient with the R271W substitution is in process. No other member of these families was diagnosed with congenital malformations.
Conclusion: Our report constitutes the first observation suggesting that mutations in HNF-1alpha may, although probably more rarely than in HNF-1beta, produce the phenotype of a single functioning kidney and genital malformation.
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A new mutation in the LMNA gene with a mixed phenotype and early senescence of cultured fibroblasts

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Background and Aims: Mutations in type A-lamins are responsible for several clinically distinct pathological conditions, including syndromes with insulin resistance and/or lipodystrophy, muscular dystrophies and premature ageing syndromes, in which major insulin resistance is present. Although the pathophysiology of these diseases remains elusive, mixed phenotypes suggest that they could represent a pathological continuum involving tissues of mesenchymal origin.
Material and Methods, Results: We report the clinical observation and fibroblasts studies from a 48-years-old woman exhibiting a new heterozygous LMNA L92F substitution. Her phenotype associated diabetes, diagnosed at age 23, partial lipodystrophy, severe insulin resistance evidenced using a hyperinsulinemic euglycemic clamp, and bilateral cataract diagnosed in her early fourties. In addition, she complained of diffuse arthromyalgia and fatigability of the four limbs. CPK were normal. Apart from distal neurogenic signs secondary to diabetic neuropathy, electromyogram showed atypical myogenic alterations of the proximal lower limbs. 24-hour ECG-monitoring, echocardiography and myocardial scintigraphy did not reveal any abnormality. Her father and paternal grand-father died from heart failure at age 46 and cardiac arrhythmia at age 64, respectively. Immunofluorescence microscopic analysis of her cultured skin fibroblasts revealed abnormalities of the nuclear shape and the distribution of lamins, similar to those previously reported in other laminopathies. Using the MTT (thiazolyl blue)-based test, we showed that the patient's cultured fibroblasts presented a decreased viability as compared with control cells. The mutated fibroblasts had an increased doubling time in culture. In addition, using the Senescence-Associated Beta-Galactosidase staining assay (which evaluates the beta-galactosidase activity at pH6, a marker of cellular senescence), we found that the mutated fibroblasts reached more rapidly a state of cellular senescence than control cells.
Conclusion: This observation reports a mixed phenotype of laminopathy with a new mutation in the LMNA gene. Despite the absence of clinical premature ageing signs in this patient, we found several features of accelerated senescence in her cultured fibroblasts. Further studies are needed to know if early senescence is a general feature of all LMNA-mutated cultured cells.
We thank the Endocrinology-Nutrition Department at the Edouard Herriot Hospital, Lyon, for providing skin fibroblasts from the affected patient. This work has been supported by the Fondation pour la Recherche Médicale, France.

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A newly detected mutation in the glucokinase gene in a Czech family with maturity-onset diabetes of the young

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Background and Aims: Glucokinase (GCK) is a key enzyme regulating glucose homeostasis. GCK is mainly expressed in the pancreatic beta-cells and in the liver but also in the selected neuroendocrine cells of the gastrointestinal tract and the brain. GCK mutations are known as a pathogenetic cause of the maturity-onset diabetes of the young type 2 (MODY2) characterized by mild, persistent fasting hyperglycaemia, low glucose-stimulated insulin secretion, autosomal dominant inheritance and early onset. These mutations are also found in gestational diabetics. The aim of our study was to assess the variability of the GCK gene in the Czech diabetic and control populations.
Materials and Methods: Screening for sequence variants in all 10 exons of the GCK gene and flanking intron regions was performed by single strand conformation polymorphism (PCR-SSCP) and/or by temperature gradient gel electrophoresis (PCR-TGGE) in patients with MODY (unrecognized type) (n=9 basic patients + 10 family members), diabetes mellitus type 2 (DM2) (n=296), gestational diabetics (GD) (n=119), healthy offspring of diabetic patients (PD) (n=127), and healthy controls without family history of DM2 (K) (n=120). The positive controls for each exon were used. DNA polymorphisms were confirmed by direct sequencing.
Results:
We found the following polymorphisms:
MODY (n=1): in exon 2 a novel heterozygous missense mutation 98T>C (GTG>GCG) which leads to amino-acid change Val33Ala.
DM2: IVS1+4T>A (n=1); exon6 645C>T (Tyr215Tyr) (n=2); exon7 789C>T (Ser263Ser) (n=1); IVS8+18G>A (n=2); IVS9+8T>C (31%); IVS9+49G>A (7,3%); IVS9+8T>C i IVS9+49G>A (1,7%).
GD: IVS1+4T>A (n=1); IVS3+9C>T (n=1); exon6 645C>T (Tyr215Tyr) (n=2); IVS8+18G>A (n=2); IVS9+8T>C (24,5%); IVS9+49G>A (9,2%); IVS9+8T>C i IVS9+49G>A (5,1%).
PD: IVS1+4T>A (n=1); exon6 645C>T (Tyr215Tyr) (n=1); IVS9+8T>C (33,3%); IVS9+49G>A (11,9%); IVS9+8T>C i IVS9+49G>A (2,4%).
K: IVS2-23C>T (n=2); exon6 645C>T Tyr215Tyr (n=1); IVS9+8T>C (35,5%); IVS9+49G>A (13,1%); IVS9+8T>C i IVS9+49G>A (0,9%).
Conclusion: We found a novel mutation Val33Ala in exon 2 of GCK gene in a patient with MODY. However, our study did not provide the evidence of GCK gene as a risk gene in the pathogenesis of diabetes mellitus type 2 and/or of the gestational diabetes in Czech population because we did not find any known GCK pathogenic mutations and any differences in the frequencies of GCK polymorphisms between Czech diabetic and nondiabetic populations.
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Phenotypic characteristics of early onset autosomal dominant type 2 diabetes in caucasians from east coast centre Italy

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Background and Aims: Some adult diabetic patients, although defined as being affected by type 2 diabetes (T2D), are indeed affected by an early onset diabetes mellitus (EOD) which is characterized by autosomal dominant inheritance resembling that of Maturity Onset Diabetes of the Young (MODY). In fact, 11 to 45% EOD families have been reported not to be linked to genes whose mutations are so far recognized as causes of MODY. Whether clinical phenotypes of MODY-gene positive and MODY-gene negative EOD patients are different is still a matter of debate. Aim of the present study was to characterize clinical phenotypes of EOD families in a Caucasian population from Italy.
Materials and Methods: Fifty-two families with a pattern of occurrence of EOD were enrolled into the study. Inclusion criteria were as follows: 1. a proband and at least one first-degree relative with T2D diagnosed before age 35 yrs; 2. three or more generations affected by T2D. Exclusion criteria were the presence of clinically evident autoimmune disease and/or systemic diseases known to impair glucose homeostasis. All subjects underwent physical and biochemical examination. Glycemic status was defined according to World Health Organization criteria. Mutational analysis of the six MODY genes has been performed by PCR, DHPLC and direct sequencing. Statistical analyses were performed using the SPSS software program version 12.0 (2003).
Results: A total of 52 families including 132 T2D patients, 6 impaired glucose tolerant, 15 with impaired fasting glucose and 123 non diabetic subjects were enrolled from 2001 to date. Five families revealed the presence of a mutation in MODY genes. One out of five was affected by MODY 1, two by MODY 2, one by MODY 3 and one by MODY 4. Three out of five mutations have never been described before (R136W in HNF-4α, P59S in GCK and 864delG in HNF-1α). EOD patients were then subdivided in two groups: MODY-gene positive (n=10) and MODY-gene negative (n=81). Comparison of clinical features revealed that patients from the latter group have older age at diabetes diagnosis (37.3 ± 11.8 vs. 23.1 ± 10, $p=<0.001$), higher BMI (29.7 ± 5.7 Kg/m^2 vs. 24.8 ± 3.2 Kg/m^2, $p=0.009$) and higher proportion of dyslipidemia (84.7% vs. 50%, $p=0.021$) and hypertension (74.7% vs 30%, $p=0.008$), thus resembling typical features of the Insulin Resistance/Metabolic Syndrome. These features, are superimposable to that of "classical" type 2 diabetic patients (age of onset 50.6 ± 10.3 years, with no evidence of autosomal dominant inheritance) recruited in the same Institution during the same period of time (BMI=30.8 ± 5.6, 86.6% of dyslipidemia and 83.5% of hypertension).
Conclusion: Our study indicates that, only a small proportion (i.e. 10%) of Caucasian EOD families from Italy are affected by mutations of MODY genes. Clinical features of MODY-gene negative EOD are similar to that of "classical" T2D. Whole genome studies will be performed on these families aimed at understanding the genetic basis of EOD.
Support: Italian Ministery of Health

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Assessment of the role of genetic variation in the transient neonatal diabetes (TNDM) region on chromosome 6q24 in type 2 diabetes: a comparative genomic and haplotype approach

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Background & Aims: Recent reports have supported the hypothesis that genes involved in monogenic forms of diabetes make excellent candidate genes for type 2 diabetes. Transient neonatal diabetes (TNDM) is a rare disorder associated with overexpression of genes at a paternally-expressed imprinted locus on chromosome 6q24, the key candidate gene being the transcription factor *ZAC*. Several type 2 diabetes linkage studies have indicated linkage to chromosome 6q22-25, in one case with evidence of over-transmission of paternal alleles. We hypothesised that common genetic variation at this TNDM region could influence susceptibility to type 2 diabetes.
Materials & Methods: Comparative genomic analysis was used to identify common variants within the *ZAC* region, from which its haplotype structure was derived.
Results: Seven SNPs with minor allele frequencies ranging from 15–46%, which captured the 5 most common haplotypes were genotyped in a large-scale case-control (n=3643) and family based (n=520 families) study. This sample size had >80% power to detect odds ratios of >1.2 for all tagging SNPs. None of the 7 SNPs nor the haplotypes formed by these SNPs were associated with diabetes in our case control study (global p values for haplotype blocks 1 & 2 respectively = 0.81, 0.74). Nor did we find evidence for over transmission of any SNP or haplotype to affected offspring (global p values for blocks 1 & 2 = 0.09, 0.17), or for a parent of origin effect (p values ranged between 0.03–1 for the 7 SNPs).
Conclusions: We conclude that common genetic variation at this locus is unlikely to influence susceptibility to Type 2 diabetes, although we cannot exclude a parent of origin effect with modest effect size.
Support: Diabetes UK

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Defining cardiovascular risk in type 2 diabetes

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Does the metabolic syndrome detect further subjects at high risk of cardiovascular death, or is a cardiovascular risk score adequate? The DECODE Study Group

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Aim: Current cardiovascular (CVD) risk scores enable the detection of subjects at high CVD risk, however it is not known whether the NCEP-ATP III metabolic syndrome might provide a useful screening method in those with a low estimated 10-year risk of CVD mortality,
Subjects and Methods: 2790 and 3324 non-diabetic men and women, 50–69 years from seven European studies participating in the DECODE Study, were followed for CVD mortality over 10 years. Hazard ratios from the Cox model were used to compare subjects according to an estimated 10-year risk of CVD mortality under/over 5% and absence/presence of the metabolic syndrome. The European SCORE project CVD risk equation was used.
Results: Overall 51% of the men and 85% of the women were identified as having a 10-year risk of fatal CVD under 5%, and for these subjects, 22% and 21% respectively had the metabolic syndrome. Over the 10 years, 118 men and 24 women died of CVD. For men at a low estimated CVD risk the hazard ratio for fatal CVD was 2.71 (1.33–5.51) for men with the syndrome ($p < 0.01$) in comparison to men without the syndrome, after adjusting for age and for the study centre; for women, the corresponding hazards ratio was 1.40 (0.43–4.50). Further adjustment for the estimated CVD risk attenuated slightly these hazards ratios. For subjects with a 10-year risk of fatal CVD over 5%, the metabolic syndrome did not provide additional discrimination. In men and women with a low CVD risk, the NCEP-ATP III cut-points for high waist circumference, identified 17% of men and 31% of women; the corresponding hazards ratios were 2.24 (1.05–4.76) ($p < 0.05$) for men and 2.28 (0.77–6.70) for women.
Conclusions: Of subjects not identified to be at a high CVD risk by a CVD risk score, men but not women with the metabolic syndrome had a significantly higher risk of fatal CVD; however, a high waist circumference provided a similar discrimination. Use of the metabolic syndrome in clinical practice is thus justified in men, but the waist circumference might provide a simpler diagnostic tool than the metabolic syndrome that requires more complicated and expensive laboratory methods.

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Circulating oxidized low-density lipoprotein and its association with carotid intimal media thickness in subjects with glucose intolerance

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Background and aim: The risk for atherosclerosis is reported to be higher in subjects with impaired glucose tolerance [IGT] compared to those with normal glucose tolerance [NGT]. However, very few studies have looked at pre-clinical atherosclerotic markers like carotid intimal medial thickness [IMT] and newer cardiovascular risk factors like oxidized LDL and its association, particularly in subjects with IGT. The aim of the present study is to examine the relation between oxidized LDL and IMT in subjects with different grades of glucose intolerance.
Methods: The following subjects: Group 1:normal glucose tolerance (NGT) (n=150); Group 2:impaired glucose tolerance (IGT)(n=150) and Group 3:type 2 diabetes (n = 150), were recruited from the Chennai Urban Rural Epidemiology Study [CURES], an ongoing population based study on a representative population of Chennai city in southern India. NGT, IGT and diabetes were defined using World Health Organisation consulting group criteria. The inclusion criteria were normal resting 12 lead ECG, absence of angina, myocardial infarction or history of any known vascular, infectious or inflammatory diseases and subjects not on statins or aspirin. Oxidized LDL was measured using ELISA assay [Mecordia, Sweden]. The intra assay precision co-efficient of variation ranged from 2.4% to 5.5% and inter assay precision co-efficient of variation ranged from 5.2% to 8.6%.

Results: Subjects with diabetes and IGT were older (DM: 53 ± 9 years, p<0.001, IGT: 51 ± 10 years, p<0.001) compared to NGT group (46 ± 10 years). Subjects with diabetes and IGT had higher values of oxidized LDL (DM: 40.2 ± 13.1 U/L, p<0.001, IGT: 34.3 ± 12.8 U/L, p<0.001) compared to NGT group (26.6 ± 16.6 U/L). IMT values were also higher in those with diabetes and IGT (DM: 0.85 ± 0.3 mm, p<0.001, IGT: 0.79 ± .16 mm, p<0.001) compared to NGT group (0.71 ± 0.12 mm). IMT showed a strong correlation with oxidized LDL [r=0.295, p<0.001], and the correlation coefficient was significant even when subjects were categorized based on the glucose intolerance [IGT:r=0.481, p<0.001, NGT:r=0.544, p<0.001], except in subjects with diabetes. Multiple linear regression analysis revealed oxidized LDL to be strongly associated with IMT, even after adjusting for age, gender, triglycerides, HDL cholesterol, LDL cholesterol and glucose intolerance status [p=0.001]. When segregated based on glucose intolerance status, oxidized LDL showed a strong association with IMT even after adjusting for age, gender, triglycerides, HDL cholesterol and LDL cholesterol in subjects with NGT [p<0.001] and IGT [p<0.001], but not in subjects with diabetes.
Conclusion: This study demonstrates that increased levels of oxidized LDL seen in Asian Indian subjects with IGT might be a contributory factor for increased atherosclerosis in these subjects.

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Effect modification by age on the cardiovascular predictivity of non-HDL cholesterol and apoB in a Mediterranean population of type 2 diabetes: the Casale Monferrato study
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Background and Aims: Studies performed in selected groups of diabetic subjects living in the United States or in Northern European countries have suggested that non-HDL-cholesterol and apoB could be more useful predictors of cardiovascular diseases than total and LDL-cholesterol. No population-based study, however, has been performed in areas at lower cardiovascular risk as in Mediterranean countries. Aims of the present analysis were: 1) to investigate the relationship between plasma lipids and mortality, independently of other cardiovascular risk factors, in a Italian population-based cohort of type 2 diabetes; 2) to assess whereas modification by age occurred.
Materials and Methods: The study base was the population-based cohort of 1565 diabetic subjects (median age 68.9 yrs) of the Casale Monferrato Study, representative of Italian diabetic subjects. The predictive roles on 11-yrs all-cause and cardiovascular mortality (ICD-9 codes 390–459) was assessed by using multivariate Cox proportional hazards modeling, which adjusted for classical risk factors (age, sex, hypertension, smoking, CHD), novel risk factors (AER, fibrinogen), HbA1c cumulative average and referring physician.
Results: 685 deaths were identified in 10890.2 person-years of observations. Decreasing trends of multiple-adjusted HRs across quartiles of total, LDL- and non-HDL-cholesterol were evident. HRs of non-HDL cholesterol in the upper vs lower quartiles were 0.79 (95% CI 0.54–1.15) for cardiovascular mortality and 0.70 (0.54–0.91) for all-cause mortality. Although apoB and non-HDL-cholesterol were correlated (r=0.49, p<0.0001), in multivariate analyses their predictive roles on cardiovascular mortality were markedly different: adjusted HRs of apoB were 1.59 (95% CI 1.10–2.92) and 1.48 (95% CI 1.02–2.14) in the upper quartiles vs. first quartile. Since interaction terms with age were significant, we performed stratified analyses by age at baseline examination (categorized by median age, <70 vs ≥70 yrs). The apparent protective role on cardiovascular mortality of high levels of non-HDL cholesterol was evident in the oldest age-group only: HRs in upper quartile 1.56 (95% CI 0.74–3.30) in age-group <70 yrs and 0.58 (95% CI 0.36–0.93) in age-group ≥70 yrs. Respective values for apoB were 2.49 (1.16–5.37) and 1.29 (0.84–1.98); for LDL/HDL, 1.36 (CI 0.60–3.07) and 1.28 (0.84–1.97).
Conclusion: Our population-based study indicates that in Mediterranean diabetic subjects apoB is a better independent predictor of cardiovascular mortality than non-HDL-cholesterol up to age 70 yrs. In subjects aged >=70 yrs, the predictive role of apoB is still evident, although lower than in age <70 yrs, whereas non-HDL cholesterol shows an apparent protective effect for both all-cause and cardiovascular mortality, probably due to the effect of confounders such as comorbidity and frailty. No effect modification by age was evident for apoA1 and HDL cholesterol, which showed decreasing trends of mortality risk by increasing of their plasma values. Our findings support the recommendation to add apoB measurement in lipid profile assessment of diabetic subjects, particularly in the elderly, in whom prediction based on total and non-HDL-cholesterol values could be misleading.

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All cause and cardiovascular mortality in diabetic subjects increases significantly with declining glomerular filtration rate - 10 year data from the South Tees Diabetes Mortality Study
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Background: Recent analysis of large cardiovascular outcome trials has shown a close association between estimated GFR (eGFR) and mortality but data in diabetes are limited. We explored the South Tees Diabetes Mortality database for a link between eGFR and mortality.
Aims: To investigate the association of eGFR with total and cardiovascular mortality in a population based cohort of diabetic subjects.
Methods: Estimated GFR was calculated using the Modification of Diet in Renal Disease(MDRD) equation in 3288 diabetic subjects(male 56%).Subjects were stratified by baseline eGFR(ml/min per 1.73 m^2) into 5 groups as recommended by the Renal Association guidelines: >90,60 to 89,30 to 59, 15 to 29 and <15. Causes of death were analysed from death certificates and coded using ICD-10 criteria. Kaplan Meier mortality estimates and hazard ratios were calculated across groups.
Results: At baseline, mean age (58.4 years; SD16.05) differed significantly between groups (p<0.001). Median follow up was 10.5 years amounting to 28342 person years. 36% of the cohort (n=1193, males, 56%) had died by 10 years. 60% of deaths were due to cardiovascular causes.Total and cardiovascular mortality increased progressively with declining eGFR. Unadjusted all cause mortality hazard ratios (95% CI) comparing groups 2-5 to group 1 were 1.57 (1.31–1.90), 3.45 (2.86–4.16), 8.86 (6.33–12.41) and 5.34 (1.70–16.76) respectively. Kaplan-Meier estimates of 10 year mortality (%) in groups 1 to 5 were: 23, 37, 81, 214 and 92 respectively (Log rank p<0.0001). Adjusted all cause mortality hazard ratios(95% CI) comparing groups 2–5 to group 1 were 1.32 (1.05–1.65), 2.75 (2.18–3.47), 7.07 (4.61–10.85) and 4.06 (1.27–12.93) respectively. A 10 ml/min decrease in estimated GFR increased the risk of death by 31%.
Conclusions: In diabetic subjects, mortality increases significantly with decreasing GFR. Prediction equations should be used routinely to stratify diabetic subjects by estimated GFR. A declining GFR identifies patients at high risk of cardiovascular mortality who might be expected to benefit from aggressive risk factor modification.

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Simultaneous dobutamine stress echocardiography and SPECT-MPI with Tc99m tetrofosmin for assessment of coronary artery disease in diabetic patients
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Background and Aims: Simultaneous dobutamine stress echocardiography (DSE) and Tc99 m tetrofosmin single photon emission computer tomography (SPECT) - myocardial perfusion imaging (MPI) for the evaluation of the coronary artery disease (CAD) in patients with diabetes were assessed for a head to head to comparison, regarding the sensitivity and specificity of the two tests.
Materials and Methods: A total of 40 consecutive subjects were studied; mean age 45+/– 8 years.All of them underwent DSE according to Standard Mayo clinic protocol and SPECT-MPI with single day stress-rest protocol. Coronary angiogram was performed within two weeks.More than 50% stenosis was taken as significant.
Results: The overall sensitivity, specificity, positive predictive value & negative predictive value of DSE for diagnosis of presence and absence of CAD were 82%, 80%, 94% and 48%. The overall sensitivity, specificity, positive predictive value & negative predictive value of SPECT-MPI for diagnosis of presence and absence of CAD were 94%, 75%, 96% and 60%. SPECT-MPI showed a significant higher sensitivity but ratively lower specificity in comparison with DSE (p value = <.01).
Conclusion: Both non-invasive methods for the detection of CAD showed a good diagnostic sensitivity. Nevertheless the SPECT-MPI showed a higher sensitivity in comparison with DSE in this group of population.
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Coronary heart disease and stroke mortality and metabolic syndrome in UK African Caribbeans and Europeans. A population based prospective cohort study

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Background and Aims: People of Black African descent worldwide have high rates of diabetes, insulin resistance, and hypertension, but despite this, appear to be protected from CHD compared to people of European descent. Little is known either about the role of the metabolic syndrome in predicting mortality in UK African Caribbeans, or how it may contribute to ethnic group differences in cardiovascular disease mortality. We studied CHD and stroke mortality in a cohort of UK Europeans and African Caribbeans and their associations with the metabolic syndrome as defined by the World Health Organisation (WHO) and the National Cholesterol Education Programme (NCEP).
Materials and Methods: Population based prospective cohort of 2,346 white Europeans (76% male) and 801 African Caribbeans (57% male) aged between 40 and 69 years and resident in London. Fasting bloods, overnight urine and anthropometrics were measured at baseline (1988–91).
Results: During the median follow-up period of 14.4 years. 100 Europeans and 10 African Caribbeans died from CHD and 26 Europeans and 16 African Caribbeans died from stroke. Age adjusted hazard ratios (HRs) for CHD mortality were 0.48(p=0.02), 0.35 (p=0.008) and 0.30 (p=0.049) for European women, African Caribbean men and African Caribbean women compared with European men (CHD mortality rate: 3.6/1000 person years). For stroke mortality, HRs were respectively 0.60 (p=0.35), 1.12 (p=0.80) and 2.44 (p=0.02) compared with European men (stroke mortality rate: 0.88/1000 person years). WHO defined metabolic syndrome was present at baseline in 19% and 9% of European men and women and in 27% and 26% of African Caribbean men and women. NCEP defined metabolic syndrome was present at baseline in 18% and 14% of European men and women and in 16% and 23% of African Caribbean men and women. Metabolic syndrome was predictive of CHD mortality in European men only (HRs: WHO: 2.52, p<0.001, NCEP:2.13, p<0.001), and of stroke mortality in African Caribbean men only (HRs: WHO: 14.2, p=0.01, NCEP:11.1, p=0.005). Ethnicity and gender differences in CHD and stroke mortality rates remained after adjustment for presence of metabolic syndrome by either or both definitions.
Conclusions: Compared with European men, European women and African Caribbeans had low rates of CHD related mortality. Stroke mortality rates were significantly higher in African Caribbean women, but not in African Caribbean men. Metabolic syndrome, by either definition, predicted CHD in European men only and stroke in African Caribbean men only. Neither definition explained ethnicity and gender differentials in CHD or stroke mortality. These findings suggest that further prospective studies are needed to elucidate the pathogenesis of ethnicity and gender differentials in cardiovascular morbidity and mortality. Such studies should include validation of ethnicity and gender specific criteria, which are both readily usable and usefully predictive, in defining the metabolic syndrome.
These analyses were supported by the British Heart Foundation. The Brent and Southall Studies were supported by the U.K. Medical Research Council and the British Heart Foundation. IFG is supported by the Heart Disease and Diabetes Research Trust.

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Incidence of stroke in type 2 diabetic patients in Italian outpatient clinics: The DAI Study

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Background and Aims: Type 2 diabetes mellitus (T2DM) is a well established risk factor for ischaemic stroke. However, little information is available on the occurrence and risk factors of stroke in T2DM patients who are regularly seen at diabetes clinics and receive standard care.
Materials and Methods: The reference population consisted of all patients visited at 201 Italian diabetes clinics between Sept 1998-June 1999. In each centre, a representative sample of the diabetic population was selected. A total of 14,431 patients from 157 centres (7211 women and 7220 men) participated in the incidence study and were followed up for 3 years. The data collected included anthropometrics and lifestyle habits, clinical history, data relevant to both microvascular (nephropathy and retinopathy) and macrovascular complications (stroke, myocardial infarction, ischaemic heart disease, coronary artery bypass, coronary angioplasty and amputations), laboratory data, and pharmacological treatment (for hyperglycaemia, hypertension and dyslipidaemia). Events were ascertained by an *ad hoc* committee. Statistical analysis was conducted using Cox Proportional Hazard Model.
Results: At baseline 2789 patients had evidence of macrovascular complications (prevalence cohort) and 11,647 were free from them (incidence cohort). During the follow-up period, a total of 153 strokes were documented. In the incidence cohort, the age-standardised incidence rates of stroke were 0.5 (per 100 person-years) (95% C.I. 0.4–0.6) in men and 0.7 (95% C.I. 0.5–0.9) in women. In the prevalent cohort, incidence rates were (1.1; 95% C.I. 0.6–1.6) in men and (1.1; 95% C.I. 0.7–1.5) in women. In men, age (HR 1.8; 95% C.I. 1.3–2.4), HbA_{1c} (HR 1.3; 95% C.I. 1.1–1.5), insulin therapy in combination with oral agents (HR 3.3; 95% C.I. 1.2-8.8) and smoking (HR 2.1; 95% C.I. 1.2-3.7) were independent predictors of first ever stroke. In women, independent predictors of first stroke were age (HR 2.1; 95% C.I. 1.6–2.9) and presence of microvascular complications (HR 1.7; 95% C.I. 1.1–2.6). In the prevalent cohort, age and previous cerebral thromboembolism were strong predictors of stroke regardless of gender.
Conclusion: In T2DM patients attending Italian diabetes clinics, age plays an important role in the occurrence and recurrence of stroke. In addition, the incidence of the first ever stroke is mostly explained by smoking, metabolic control and combined (insulin plus oral agents) therapy in men and by presence of microvascular complications in women. History of previous stroke at baseline is a strong risk factor for recurrence.

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Remaining risk for acute stroke in patients treated for hypertension and type 2 diabetes in primary care: Skaraborg hypertension and diabetes project

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Background and Aims: To investigate the independent risk of acute stroke in subgroups of patients with hypertension and type-2 diabetes in primary care.
Materials and Methods: Patients with hypertension only (n=695), type-2 diabetes only (n=181), or both (n=240), who consecutively attended an annual control in primary care in Skara, Sweden, 1992–1993 were evaluated for CVD risk factors and enrolled for this study. Subjects without hypertension or type-2 diabetes (n=824) who participated in a population survey served as controls. Possible events of acute stroke through 2002 were validated using hospital records and death certificates.
Results: During a mean follow-up time of 8.4 years 190 first events of acute stroke, fatal or non-fatal, were ascertained. Risk factor levels were generally higher in all patient categories than in controls. In men stroke risk was significantly increased in those with both type-2 diabetes and hypertension: HR 4.2 (95%CI 2.1–8.4), type-2 diabetes alone 3.3 (1.5–7.0), or hypertension alone 2.8 (1.5–5.3) (controlling for age, total cholesterol, current smoking, BMI, and physical activity). Corresponding findings in women with type-2 diabetes only were 2.9 (1.5–5.8), and in those with both conditions 2.4 (1.2–4.7). However, in women without diabetes a significant risk associated with hypertension was seen first when subjects were truncated at age 85 years. There were too few fatal strokes for conclusive results.
Conclusion: A considerable risk of acute stroke remains in patients with hypertension or type-2 diabetes treated in primary care. As insufficient risk factor control seems to be contributing stricter multiple risk factor interventions should be implemented.
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Correlates of the metabolic syndrome and mortality in type 2 diabetes

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A haplotype combination in endothelial nitric oxide synthase gene is associated with metabolic syndrome and related parameters

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Background and Aims: Nitric oxide (NO) is synthesised by the nitric oxide synthase (NOS) enzyme. Three isoforms of this enzyme have been identified. Reported studies in humans and rats show that inhibition of endothelial NOS (NOS3) attenuates insulin dependent glucose uptake in several insulin sensitive tissues, indicating that glucose uptake via insulin signalling pathways is NO dependent. Moreover, NOS3 knockout mice are hypertensive, hyperinsulinaemic and dyslipidaemic, suggesting that a deficiency in the NO synthesis might be associated with metabolic syndrome (MS)-related alterations. The aim of this study was to investigate the relationship between NOS3 gene haplotypes, MS and MS-related variables.
Materials and Methods: 738 unrelated subjects (35–74 years, 306 men), from a cross-sectional population-based epidemiological survey in the province of Segovia (Spain) were studied. The whole population was genotyped for three SNPs (rs2070744, IVS11-30, rs3800787) at the NOS3 gene, and haplotypes were re-constructed. Genetic variants were screened using an adaptation of the Fluorescence Polarization template directed incorporation method. Alleles at each genetic locus were code "1" (wildtype) or "2". Each number within the haplotype was ordered according to its genomic location. Anthropometric/biochemical parameters were waist circumference, systolic and diastolic blood pressures, fasting glucose, fasting insulin, HDL-cholesterol, and triglycerides. MS was defined according to ATP III criteria. Insulin resistance was estimated by HOMA IR. Haplotype-phenotype associations were evaluated using the THESIAS software (http://www.genecanvas.org). This method allows the estimation of haplotype frequencies and haplotype effects by comparison to a reference group (referent), taken here as the most frequent haplotype. Haplotype effects are expressed as the difference between the phenotypic mean for haplotype 212 relative to the referent phenotypic mean.
Results: Allele frequencies: 1) locus rs2070744 (2/1): 0.53 / 0.47; 2) locus IVS11-30 (1/2): 0.48 / 0.52; 3) locus rs3800787 (1/2): 0.64 / 0.36. All genotypes were in Hardy-Weinberg equilibrium ($p>0.05$). Haplotype 212 was associated with a higher prevalence of MS (adjOR 1.80, 95% CI 1.15–2.85, $p = 0.011$). Furthermore, we found that subjects with the haplotype 212 had 35.7% higher values of HOMA IR ($p = 0.040$), 23.73% higher circulating triglyceride levels ($p = 0.026$) and 36.28% higher proinsulin levels ($p = 0.015$) than those with the most frequent haplotype (121).
Conclusion:Our findings suggest that variations at the NOS3 gene, and specifically the haplotype 212, might affect the risk of MS, insulin resistance and hypertriglyceridemia.
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Increased waist circumference and the presence of the metabolic syndrome predict the incidence of microalbuminuria in an adult population. The D.E.S.I.R Study

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Background and Aims: A cross-sectional association between microalbuminuria, insulin resistance and the metabolic syndrome has been reported. However, metabolic determinants of the occurrence of microalbuminuria have not been prospectively investigated in non-diabetic cohorts. The aim of our study was to investigate the relation between the components of the metabolic syndrome (NCEP-ATP III definition) and the incidence of microalbuminuria after 6-years.
Materials and Methods: We studied 2377 adults subjects from the French DESIR cohort without microalbuminuria (UAE <20 mg/l) or diabetes at baseline. Albuminuria was assessed on a single morning sample at inclusion and at 6 years.
Results: At 6 years, 186 individuals (7.8%) had developed elevated microalbuminuria (≥ 20 mg/l). In univariate analysis, variables significantly associated with incident microalbuminuria in both sexes were BMI, waist circumference (WC), mean arterial BP. In men only, the incidence of microalbuminuria was also associated with baseline fasting plasma glucose, fibrinogen, HbA1c, insulin levels and the HOMA-IR index. In both sexes, the percentage of subjects microalbuminuric at 6 years was significantly higher in those with a waist circumference >102/88 cm at baseline as compared to those with normal WC (30.1% vs 12.5%, $p<0.0001$ for women, 12.6% vs 5.5%, $p=0.04$ for men). Subjects with the metabolic syndrome at baseline were more likely to be microalbuminuric at 6 years than those without the metabolic syndrome (12.0% vs 4.1%, $p=0.001$ for women, 10.7% vs 5.8%, $p=0.04$ for men). In multivariate logistic analysis, in men, waist circumference as a continuous variable (OR: 1.35, 95% CI: 1.1–1.7, $p=0.006$) or a WC >90 cm (OR: 1.61, 95% CI: 1.1–2.5, $p=0.03$) were predictive of incident microalbuminuria, independently of age, BMI, fibrinogen, blood pressure level, antihypertensive treatments, HbA1c, glycaemia, and HOMA index. In women, the independent predictive variables were a WC >90 cm (OR: 3.20, 95% CI: 1.4–7.1, $p=0.004$) and the presence of the metabolic syndrome at baseline (OR: 2.32,95% CI: 1.0–5.4, $p=0.04$).
Conclusion: These findings show that abdominal adiposity is strongly related to the development of microalbuminuria in a non-diabetic population, suggesting the potential role of visceral adipocytes in the pathogeny of microalbuminuria and the importance of screening for microalbuminuria among individuals with modestly elevated waist circumference (>90 cm).

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High prevalence of diabetes mellitus and metabolic syndrome in the BEST study ("Belgian Evaluation of Screening and Treatment of high risk patients based on waist and age")

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Background and Aims: Abdominal adiposity is considered as a major risk factor for type 2 diabetes, metabolic syndrome (MetS) and cardiovascular diseases. The objective of this study was to determine cardiovascular risk factors in a Belgian middle-aged (40–75 years) population without any cardiovascular history, selected upon a moderately increased waist circumference (WC: male ≥ 94 cm, female ≥ 80 cm) as simple unique clinical criterion. The present analysis focuses on the prevalence and characteristics of type 2 diabetes and MetS in this population.
Materials and Methods: Consecutive patients were recruited by 619 GPs during spring 2004. A central lab analysed fasting blood samples for glucose, total cholesterol, HDL-cholesterol, and triglycerides. Complete data were obtained on 8587 patients (47% females; 24% smokers). MetS was defined according to NCEP-ATP III criteria.
Results: The prevalence of diabetes (fasting glucose ≥ 7 mmol/l) averaged 11% in men with WC 94–101 cm and 9% in women with WC 80–87 cm, but almost doubled in men with WC ≥ 102 cm (21%) and in women with WC ≥88 cm (19%). The prevalence of MetS was rather low in men (6%) and women (3%) with moderate WC increase, but rose markedly in individuals with larger WC increase (37% and 29%, respectively). Among non-diabetic patients (NDP), 25% had ≥ 3 components of MetS and 31% had 2 components. A total of 1527 individuals (18%, 14% treated, 4% not treated) were diabetic patients (DP), with fasting glucose level averaging 8.5 mmol/l (38% had a family history of diabetes vs 15% in NDP). As compared to NDP, DP were slightly older (61 vs 58 yrs), were heavier (BMI: 31.8 vs 30.1 kg/m^2), had a larger waist (104 vs 97 cm for women and 111 vs 106 cm for men), and took lipid lowering drugs (33 vs 23%) and antihypertensive agents (67 vs 44%) more frequently. About 89% of DP reported to have almost no physical activity (as compared to 83% in NDP). Mean total cholesterol (5.61 vs 5.97 mmol/l) and calculated LDL cholesterol (3.31 vs 3.67 mmol/l) levels were slightly lower in DP than NDP, but still remained above targets: 84% had total cholesterol ≥ 4.5 mmol/l (175 mg/dl) and 78% had LDL cholesterol ≥ 2.6 mmol/l (100 mg/dl). Lower HDL cholesterol (1.34 vs 1.50 mmol/l) and higher triglyceride (2.25 vs 1.81 mmol/l) levels were observed in DP than in NDP. Mean systolic (SBP: 139 vs 135 mmHg) and diastolic (83 vs 82 mmHg) blood pressure values were slightly higher in DP than in NDP. Furthermore, 82% of DP had SBP above specific target in the diabetic pop-

ulation (≥130 mmHg), contrasting with 45% of NDP having SBP ≥140 mmHg (target in the general population). On average, only 30% of drug-treated subjects had total and LDL cholesterol levels below the targets, and this was also the case for SBP. Finally, in the NDP population, total risk ≥5% for dying from cardiovascular disease in the coming 10 years (estimated with the European SCORE chart adapted for Belgium) was more than 40% in men and more than 20% in women.
Conclusion: Waist measurement is an easy and inexpensive tool to detect individuals at high risk for metabolic syndrome, diabetes mellitus, and with a variety of modifiable risk factors in general practice. In this population, there is a clear need for improved management of cardiovascular risk factors, especially lipid profile and blood pressure, in patients with type 2 diabetes in particular.

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Association between insulin resistance and intraocular pressure: the DESIR Study
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Background and Aims: Elevated intraocular pressure has been associated with hypertension, diabetes and other metabolic abnormalities. We examined the association of IOP with insulin resistance, to determine if insulin resistance is an independent predictor of elevated IOP, to assess the association of clinical characteristics with IOP and to explore possible mechanisms for the associations.
Materials and Methods: The French Epidemiological Study on the Insulin Resistance Syndrome (DESIR) performed detailed ocular studies on a subpopulation of 700 participants. Participants under went standardised examinations including tonometry, blood pressure anthropometric and biochemical measures. Insulin resistance was determined using the homeostasis model assessment (HOMA-IR).
Results: The mean age of participants was 47 years and 47% were male. Intraocular pressure was significantly higher among those with diabetes and impaired fasting glucose compared to those with normal glucose tolerance (means (SD), 17.8 mmHg (3.9) v 15.8 mmHg (3.7) v 14.8 mmHg (3.5) overall p<0.001). Multiple linear regression identified HOMA-IR, gender, smoking and cholesterol as independent factors associated with higher IOP. These associations remained after exclusion of those with diabetes mellitus. Standardised β coefficients between IOP and HOMA-IR, gender, smoking and cholesterol were 0.111 (p=0.004), -0.153 (p=<0.001), 0.082 (p=0.034) and 0.090 (p=0.022) respectively. Separate models assessing the associations between IOP and each of systolic blood pressure, family history of glaucoma, triglycerides, creatinine, BMI and cholesterol were non-significant after multivariate adjustment for age, gender and HOMA-IR.
Conclusion: This is one of the first studies to assess the association of IOP with insulin resistance. Insulin resistance was independently and positively associated with higher IOP. Insulin resistance may be an important factor in the development of elevated IOP.

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Metabolic syndrome and psychosocial precarity
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Background and Aims: Precarity and unfavourable social and economical conditions are often implicated in the development of obesity. In a previous study we showed that the prevalence of obesity in our geographic area (Seine Saint-Denis) was the highest among non working women. The aim of this study was to examine in the same area the relationships between the metabolic syndrome (MS) and precarity.
Materials and Methods: Among the people who had a health assessment offered by the national health insurance system in our center from september 2003 to june 2004, we selected 6378 subjects aged 16–91 years, without known diabetes and free of lipid lowering treatments. MS was defined according to NCEP/ATPIII criteria, and the precarity score consisted of 11 validated questions which quantified psychosocial precarity risk.
Results: MS was present in 10.8% of the population (8.6% of men and 12.9% of women), with a significant influence of age (p < 0.0001). The prevalence of MS differed significantly according to socioprofessional categories. It was maximal (19%) among retired subjects, 11.5% of unemployed people; it was the lowest among executive people and independent professionals (2.8%). MS was significantly higher among the subjects with a high psychosocial precarity score (>40 on a scale of 100) than in those with a score <40 (13.6% vs 9%; p <0.0001). The logistic regression showed that MS was significantly and independently associated with age, gender (women/men: OR = 1.806[95 CI: 1.516–2.152; p < 0.0001), socioprofessional categories, and the psychosocial precarity score (OR: 1.368 [95CI: 1.141–1.641]; p <0.001).
Conclusion: This study shows that socioprofessional category and psychosocial precarity are independent determinants of MS, and strongly suggests that preventive efforts should be concentrated in particular in unemployed women over 50 years.

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Abnormal hepato-biliary function and the metabolic syndrome
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Background and Aims: Diabetes, obesity and the metabolic syndrome (MS) are associated with abnormal liver function and non-alcoholic steatohepatitis. We have tested whether MS, defined by ATP3 criteria, is associated with abnormalities in other markers of hepatobiliary function.
Materials and Methods: All residents aged 25+ years from houses randomly selected from residential lists across 8 rural Victorian towns were visited, and then invited to attend for a 75 g oral glucose tolerance test (WHO criteria), along with questionnaire completion, anthropometric and blood pressure measurement. Blood samples were also taken for lipids and liver function tests. Overall, 2376 houses were selected and visited between June 2001 and March 2003. Household participation rate in the initial visit was 70.3%.
Results: Subject participation in the screening study was 61.3% (n=1454) of whom 56% (815) were women. The age standardized prevalence of MS was 25.3% among men and 20.8% among women. Men and women with MS were older, exercised less, watched more TV and ate less fatty food. Among men, those with MS had higher concentrations of ALT (27.9 ± 19.1 vs 36.0 ± 23.2 IU/l, p<.001), GGT (36.0 ± 31.4 vs 50.6 ± 61.0 IU/l, p<.001), ALP (71.0 ± 20.8 vs 77.7 ± 23.9 IU/l, p<.001) and bilirubin (73.5 ± 4.5 vs 74.4 ± 4.1, p=.012). Among women, those with MS also had higher concentrations of ALT (19.8 ± 14.1 vs 23.6 ± 17.0 IU/l, p=.002), GGT (26.1 ± 35.8 vs 35.7 ± 27.7 IU/l, p=.001), ALP (69.7 ± 28.7 vs 79.4 ± 25.9 IU/l, p<.001) and bilirubin (73.4 ± 4.6 vs 74.2 ± 4.7, p=.046). These differences remained after adjusting for age, socioeconomic status and alcohol intake. The proportions with an elevated ALT (>38 IU/l) among those without and with MS were 17.8% vs 33.5% (men) and 5.8% vs 10.8% (women) respectively. There were no differences in AST between those with and without MS. After adjusting for confounders in men and women, the number of components of MS correlated positively with ALT (r=0.161, p<.001; r=0.111, p=.002), ALP (r=0.100, p=.013; r=.083, p=.020), GGT (r=.125, p=.002; r=.130, p<.001) respectively. Blirubin correlated among men but not women (r=.093, p=.022). The markers of hepato-biliary function correlated variably with individual components of MS.
Conclusion: We conclude that the steato-hepatitis associated with MS, may involve wider metabolic disruption than originally proposed.

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Unexpected high cancer-related mortality in type 2 diabetes in primary care. Six years follow-up on mortality in the ZODIAC study
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Background and Aims: To longitudinally investigate cause specific mortality and associated factors in patients with type 2 diabetes mellitus (T2DM) treated in primary care.
Materials and Methods: A primary care based cohort (the ZODIAC study) of 1145 T2DM from the area around Zwolle in the Netherlands was followed up for 6 years (1998–2004). Data on mortality were gathered from hospital records and from general practitioner's patient files. The population of the eastern part of the Netherlands in 1998 was used as reference in calculating the standardised mortality ratio's (SMR). Baseline characteristics for factors associated with all cause, cardiovascular and cancer-related

mortality were analysed using Cox regression and expressed as Hazard Ratio's with 95% CI's. Factors associated with cancer mortality were assessed using Cox regression with a limited numbers of variables.
Results: There were 335 deaths (29%) in the study population during a median follow-up period of 5.8 years. From one person data on life status could not be obtained. From 20 patients (6%) the cause of death could not be traced. Biggest contributors to mortality were cardiovascular and cancer death with 48% and 22%, respectively. The SMR for all cause, cardiovascular and cancer mortality compared to the reference population were significantly raised (SMRs 1,86 (CI 1,66–2,06), 2,24 (CI 1,91–2,61), and 1,38 (CI 1,07–1,75), respectively). Women had a higher SMR than men (2,16 (CI 1,86–2,50) versus 1,76 (CI 1,49–2,06)). Variables associated with total mortality were; older age, male gender, poor glycemic control, hypertension, cardiovascular disease and microalbuminuria. Variables associated with cardiovascular mortality were; older age, male gender, poor glycemic control, cardiovascular disease, reduced renal function, and microalbuminuria. Variables associated with cancer mortality were; old age and a BMI lower than 30.
Conclusion: As expected, cardiovascular disease is the biggest contributor to mortality in T2DM in patient treated in primary care, and still much elevated despite efforts to improve prognosis through intensive treatment of cardiovascular risk factors. Unexpectedly, cancer mortality was also raised in our cohort and obesity was inversely associated with cancer mortality. A follow-up study was started in a larger population group in order to gain more insight in this phenomenon.

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All-cause mortality is increased accross glucose tolerance categories in a national Australian population-based study (AusDiab)
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Background and Aims: Diabetes is known to increase mortality, but there are few data from nationally representative cohorts which incorporated an oral glucose tolerance test (OGTT). The aim of this analysis was to describe the all-cause mortality associated with glucose tolerance status from AusDiab, a nationally representative population-based survey of 11,247 Australian adults aged ≥25 years.
Materials and Methods: Baseline measurements were obtained between 1999 and 2000. Glucose tolerance status was determined according to a 75 g OGTT and from self-reported physician diagnosis of diabetes. In 2004, vital status was ascertained by linking the AusDiab cohort to the National Death Index. Cox's proportional hazards model was used to determine the association between glucose tolerance status and all-cause mortality, after adjusting for age, gender, fasting lipids, blood pressure, waist-hip ratio, smoking and previous cardiovascular disease.
Results: Over a median follow-up of 4.2 years, there were 257 deaths (157 males and 100 females). Of those with normal glucose tolerance, 1.3% died over this period. Deaths in other groups were as follows: known diabetes – 9%; newly diagnosed diabetes – 6%; impaired glucose tolerance – 4%; and impaired fasting glucose – 3%. After adjusting for confounders, the hazard ratio (HR) for all-cause mortality in people with known diabetes was 2.0 (95%CI 1.4–3.1), compared to individuals with normal glucose tolerance. Mortality risk was also increased in people with newly diagnosed diabetes (HR 1.6 95% CI 1.0–2.5), impaired fasting glucose (HR 1.9 95%CI 1.2–3.1) and impaired glucose tolerance (HR 1.6 95% CI 1.1–2.2).
Conclusion: The results of this study confirm that individuals with previously diagnosed diabetes and with lesser degrees of glucose intolerance are at an increased risk of mortality, even over a relatively short 5-year follow-up period.

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Sex difference in cardiovascular mortality in diabetic subjects with and without metabolic syndrome
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Background: Most studies have found that diabetes (DM) eliminates the protective effect of female sex on the risk of cardiovascular disease (CVD), but others have shown that such an effect does not exist when adjusted for classic risk factors such as age, hypertension, total cholesterol and smoking.
Aim: To study the sex difference in CVD mortality in diabetic subjects with and without metabolic syndrome (MetS) using a modified WHO 1999 definition (without microalbuminuria).
Study population and methods: 4643 European men and 5528 European women from 8 DECODE cohorts aged 40–88 years were included in the data analysis. The median follow-up was 8.8 years. Cox regression analysis was performed to estimate the hazard ratio (HR) for CVD mortality separately for men and women and an interaction of sex with DM and MetS was made to test the sex difference. Newly diagnosed DM was defined according to fasting plasma glucose ≥7.0 mmol/l and/or 2-hour plasma glucose ≥11.1 mmol/l in subjects without a prior history of DM.
Results: CVD mortality (per 1000 person-year) was 4.0, 6.8, 8.7 and 9.4 in men and 1.3, 10.6. 3.2 and 5.7 in women in subjects DM- & MetS-, DM+ & MetS-, DM- & MetS+ and DM+ & MetS+. The multivariate adjusted HR was higher for diabetic women than for diabetic men in the absence of the MetS, but the difference decreased in the presence of the MetS (table 1). The interaction of sex with DM was significant (x^2=6.62, 1 df, p=0.01), whereas the interaction of sex with MetS (x^2=0.30 1df, p=0.58) and that of DM with MetS (x^2=0.16, 1df, p=0.69) were not statistically significant.
Conclusion: Although overall absolute CVD mortality was much higher in men than in women, mortality as well as the relative risk of CVD death was higher in diabetic women than in diabetic men in the absence of the MetS. Once MetS developed, the difference in CVD mortality between diabetic men and women disappeared. MetS is an important confounding factor when studying the sex-difference between diabetic men and diabetic women.

	Not Metabolic Syndrome		With Metabolic Syndrome	
	Non-diabetic	Diabetic	Non-diabetic	Diabetic
no. death/total				
Men	109/3259	3/56	82/1114	17/214
Women	48/4292	7/80	27/971	9/185
Hazard ratio adjusting for age, centre, cholesterol and smoking				
Men	1	0.86 (0.27–2.73)	1.97 (1.47–2.64)	2.00 (1.19–3.35)
Women	1	4.31 (1.93–9.64)	1.66 (1.02–2.70)	2.05 (0.99–4.26)

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Genetics of type 1 diabetes: the major players

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Y-chromosome phylogenetic analysis to dissect type 1 diabetes genetic heterogeneity

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Background and Aims: Type 1 diabetes (T1D) displays genetic heterogeneity, where contribution of certain loci to heritable disease susceptibility is different in various patient groups. Given the unique Finnish population structure, especially in the late settlement area, it is expected, that certain disease alleles are enriched in genetically related patient clusters. Using Y chromosome phylogenetic analysis we aimed to define such patient clusters and to identify locus/allele heterogeneity at various T1D loci in those clusters.
Materials and Methods: Fathers in 575 nuclear families and 120 ASP families with children with T1D collected from Finland were studied. Four Y chromosome short tandem repeats (STRs) from the non-recombining region of the chromosome (DYS389, DYS392, DYS394, DYS390) were analysed using capillary electrophoresis. The phylogenetic analysis of Y-STRs was performed with the reduced median network algorithm (Network 4.1). For family-based linkage and association studies families were assigned to the Y-STR lineage the father carried. All families were typed for HLA DRB1-DQB1-DQA1, insulin gene (-23) HphI and CTLA4 CT60 variants. In addition 29 microsatellites were analysed on chromosome X. Pairwise genetic distances, gene diversity, analysis of molecular variance (AMOVA) were analysed using the Arlequin 2.0 package. Transmission distortion and linkage was tested using standard methods.
Results: No association of Y-STRs with T1D was found. The phylogenetic analysis revealed two clearly separated Y-STRs haplotype clusters (C2 and C13) that confirmed population substructure within the T1D family material (AMOVA $p<0.0001$). The C2 Y-STR variants were more common among those living in eastern Finland (late settlement) with a clearly reduced Y-STR diversity (0.69) compared with those living in the southern regions (0.89; Fst $p<0.00001$). We found no difference in the Y-STR haplotype frequencies between patients carrying different HLA, insulin gene or CTLA4 haplotypes. However, in the family set assigned to the C2 Y-STR cluster ($n=38$), the DXS8076 marker showed a multipoint lod score of 1.3. The TDT test confirmed the disease association of this chromosome region in this cluster (global p value at DXS8076: 0.0006). In contrast, in those families who belonged to cluster 13, or other Y-STR haplotypes, no linkage or association was detected.
Conclusion: The data indicate genetic heterogeneity of the Finnish type 1 diabetes population with the presence of two major clusters of male lineages that originate from different founder events. In addition, we detected disease gene linkage heterogeneity on chromosome X between Finnish T1D families who belong to these phylogenetically distinct Y chromosome lineage clusters. This study indicates the importance of genetic characterisation of population substructure when mapping genes for polygenic disease.
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The antigenic motif of the type-1-diabetes resistant HLA-DQA1*0501/B1*0301 allele compared to the neutral allele DQA1*0301/B1*0301

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Background and Aims: To distinguish between the motifs for two HLA-DQ7 alleles with identical β-chains, conferring resistance to type-1 diabetes, (DQA1*0501/B1*0301) and the other neutral/mildly protective (A1*0301/B1*0301), we modelled their 3-dimensional structure to verify motifs obtained experimentally. The peptides used were known to bind from previous studies. Knowledge of the true motifs might point out mechanisms of resistance regarding the known protein auto-antigens.
Materials and Methods: Homology models of the two DQ7 alleles were generated based on the structures of HLA-DQ8 (A1*0301/B1*0302) and HLA-DQ2 (A1*0501/B1*0201). DQA1*0301/B1*0301 was generated from the structure of DQA1*0301/B1*0302 (DQ8), and DQA1*0501/B1*0301 from a hybrid base molecule by replacing the alpha chain and the peptide antigen from the structure of DQ8 to that of DQ2. Peptide extraction and sequencing from cells expressing the DQA1*0301/B1*0301 molecule were performed as previously described. Different residues were tested based on the physicochemical properties of the particular anchoring pockets (p). Energy minimisation showed which residues fitted best in specific pockets.
Results: The DQA1*0301/B1*0301 allele shows preference for acidic and secondarily aliphatic residues at p1, small aliphatic at p4, larger aliphatic at p6, not specific at p7 and small aliphatic/small polar residues at p9. By contrast, the DQA1*0501/B1*0301 allele shows preference for aliphatic and aromatic residues at p1, small aliphatic at p4, aliphatic at p6, non-specific at p7 and small-medium aliphatic/small-medium polar but uncharged residues at p9. Thus the same peptide would not fit well, nor bind with high affinity, in the grooves of these two alleles in the same register. The results of modelling and peptide extraction are in agreement with each other. The motif differences can be attributed to the 10 amino-acid differences between the two α-chains. Inspection of peptide sequences from the known auto-antigens showed three sequences that could bind well to the DQA1*0501/B1*0301 allele (insulin B11-19 (all sequences given as core nonamers), GAD65 245-253 and 561-569). These are overlapping to sequences from known auto-antigenic peptides, that bind to HLA-II susceptibility alleles, and to 2/3 of which T cell lines or clones have been established in patients (insulin B13-21, restricted to DQ8 and GAD65 557-565, restricted to DR401/404/405, GAD65 253-261 binding to DQ8). We also verified the fulfilment of the DQA1*0501/B1*0301 motif by two peptides from the insulin A chain (2–10 and 8–16). The insulin A4-12 sequence fulfils the DQA1*0301/B1*0301 motif.
Conclusion: Our results are consistent with determinant stealing as a mechanism of protection from type 1 diabetes by the DQA1*0501/B1*0301 allele, versus the diabetes-prone DQ8 and DR4 alleles for given diabetes epitopes. There are few sequences near the above epitopes fulfilling the motif of the DQA1*0301/B1*0301 allele, so there is little protection by this allele regarding these epitopes via determinant stealing.
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Islet antibodies more important than HLA-DQB1 genotypes in the classification of type 1 diabetes at diagnosis among young adults

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Background and Aims: During recent years, several studies have shown the difficulties to separate type 1 and type 2 diabetes if only clinical judgment is considered. We therefore assessed HLA-DQB1 genotypes, islet antibodies, fasting plasma C-peptide, and clinical features in a 5-year cohort of incident diabetic patients. The aim of this study was evaluation the value of HLA-DQB1 genotypes in relation to islet antibodies, fasting plasma C-peptide and clinical features in the classification of diabetes among young adults.

Materials and Methods: A 5-year cohort of patients with young adult (15–34 years of age) onset of diabetes in the Diabetes Incident Study in Sweden (DISS) was investigated. Among 2018 included patients, 1395 of whom were classified as with type 1, 366 as with type 2, and 257 as with unclassifiable diabetes by their reporting physician. At diagnosis, blood samples were assessed for islet antibodies (ICA, GADA, IA-2A) and HLA-DQB1 genotyping. Fasting plasma C-peptide (F-P-C-peptide) was measured 8–20 weeks after diagnosis (<0.25 nmol/l = values below the lower range).
Results: Risk HLA-DQB1 genotypes (02/0302; 21% vs. 6%, 0302/X; 22% vs. 4%, and 0302/0604; 6% vs. 2%) were increased in patients compared with controls. Islet antibodies (Ab+) were found in 83% with type 1, 23% with type 2, and in 45% with unclassifiable diabetes. Frequencies of risk HLA-DQB1 genotypes were significantly (P<0.0001) increased among type 1 Ab+ (classical type 1) diabetic patients compared with unclassifiable Ab- (60% vs. 21%), type 1 Ab- (60% vs. 28%), and type 2 Ab- (60% vs. 20%) diabetic patients. Conversely, frequencies of risk HLA-DQB1 genotypes were significantly decreased in type 2 Ab- (classical type 2) diabetic patients compared with unclassifiable Ab+ (20% vs. 67%), and type 2 Ab+ (20% vs. 51%) diabetic patients. Hence, Ab+ and not the clinical classification were associated with HLA-DQB1 risk genotypes. In the complete material, irrespective of clinical classification, risk HLA-DQB1 genotypes, young age at onset (<25 year) and low BMI (<25 kg/m^2) were significantly (P<0.0001) associated with islet antibodies. However, islet antibodies and BMI (<25 kg/m^2) but not HLA DQB1 risk genotypes, were significantly (P<0.0001) associated with low P-C-peptide concentrations (<0.25 nmol/l). Loss of weight before diagnosis of diabetes and insulin treatment were also (P<0.0001) associated with islet antibodies and low P-C-peptide (<0.25nmol/l). The incidence of type 1 diabetes using a strict clinical classification was 12.5/100,000 per year as compared with 14.2/100,000 per year when Ab+ was considered.
Conclusion: Irrespective of clinical classification, islet antibodies were associated with risk HLA-DQB1 genotypes. However, only islet antibodies were associated with the classical type 1 diabetes phenotype (young age, low BMI, weight loss, and insulin treatment). Similarly, islet antibodies but not risk HLA-DQB1 genotypes were associated with low F-P-C-peptide concentrations (<0.25 nmol/l). At diagnosis, islet antibodies are more important than HLA-DQB1 genotypes, F-P-C-peptide and clinical classification for the prediction of type 1 diabetes amongst young adults.

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Genetic interaction between protein carboxyl methyltransferase (PCMT) and SUMO 4 and HLA in the susceptibility for type 1 diabetes
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Background and Aims: PCMT is an enzyme which recognises and repairs posttranslational isoaspartyl-formation in proteins. An increase in its activity is associated with a reduction in apoptosis in rat astroglial cells. We have recently proposed *PCMT* as a biologically plausible gene involved in the development of type 1 diabetes. In addition, a functional variant in *SUMO4* (163 A>G), also involved in apoptosis, has recently been shown to be associated with type 1 diabetes. Both these genes are located in a region previously proven to be linked to type 1 diabetes (IDDM5).
The aim of this study was to assess the combined effect of *PCMT* and *SUMO4*, and *PCMT* and HLA on the risk of type 1 diabetes.
Materials and Methods: A total of 248 Danish families with type 1 diabetes (1097 individuals) were genotyped for the functional variant in the *SUMO4* gene (Tetra-primer ARMS-PCR) and a variant in the promoter region of *PCMT* (–509 C>G; RFLP) which was in complete linkage disequilibrium with a functional variant in exon 5 (358G>A) previously shown to be associated with higher enzyme repair activity. A transmission disequilibrium test (TDT) was performed to evaluate the association of the variants in both genes with type 1 diabetes. In addition, *PCMT* data was stratified according to the different risk genotypes in *SUMO4* and HLA. DR3/DR4 was defined as high-risk HLA.
Results: The variant of *SUMO4* was transmitted to 50.9% of the affected offspring (p=0.77). The variant haplotype of *PCMT* was transmitted to 46% of the affected offspring (p=0.18) and to 53% of the non-affected offspring (p = 0.099 vs. transmission to affected). When stratified according to *SUMO4* genotypes, no association was found between the *PCMT* variation and type 1 diabetes in the groups of families where the proband had low-risk genotype AA (p=0.83) or AA or AG (p=0.47). However, in the group of families where the proband had genotype AG or GG (high-risk, dominant model), the *PCMT* variant haplotype was transmitted to 95 (45.5%) of the affected offspring (p=0.19) vs. 79 (55.2%) of the non-affected offspring (p=0.069 vs transmission to affected offspring). In the group of families where the proband had genotype GG (high-risk, recessive model), the *PCMT* variant haplotype was transmitted to 24 (40.7%) of the affected offspring (p=0.15). When stratified according to HLA genotype, only in the non-high risk group (genotypes other than DR3/DR4) was there a trend towards an uneven transmission of the *PCMT* variant, to 70 (43.8%) of the affected offspring (chi-square 2.500, p= 0.11)
Conclusion: *PCMT* may be involved in the development of type 1 diabetes in certain risk groups defined by other genes involved in immunity/apoptosis.

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Cytotoxic T-lymphocyte antigen 4 (CTLA-4) and HLA haplotype interaction in type 1 diabetes susceptibility
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Background and Aims: Many gene regions have been associated with susceptibility to type 1 diabetes (T1D), but the main genetic contribution to T1D is the major histocompatibility complex (MHC) on the short arm of chromosome 6.
The cytotoxic T-lymphocyte antigen 4 gene (CTLA-4) on chromosome 2q33 has been associated with T1D and other autoimmune diseases in different populations. Thus, we analysed the role of four CTLA-4 gene variants (+49A/G, -318C/T, -1661A/G and the 24MH30G/C) polymorphism as haplotypes and their interaction with HLA risk (HLA-DR3-DQ2, DR4-DQ8) in German simplex T1D.
Materials and Methods: Families with one offspring affected with T1D (n=288) were genotyped for the +49 polymorphism, (n=242) for the -318 polymorphism, (n=196) for the -1661 polymorphism and (n=167) for the 24MH30 polymorphism and correlated with the presence or absence of HLA risk haplotype (HLA-DR3-DQ2, DR4-DQ8). Analysis of the data was performed using haploview/ETDT.
Results: The CTLA-4 variants +49G (144/97; *P*= 0.0025), 24MH30G (77/48; *P*=0.0095), -318C/+49G (120.2/73.3; *P*= 0.0008) were overtransmitted, while the -318C/+49A (76.8/129.7; *P*= 0.0002), -1661G/-318C (21.1/44; *P*= 0.0045), -1661G/-318C/+49A (15.1/37.3; *P*= 0.0022) were undertransmitted. The combination CTLA-4 318C/+49G and HLA risk haplotype conferred more suceptibilty than 318C/+49G alone.
Conclusion: The combined transmission of CTLA-4 and HLA risk haplotypes enhance genetic susceptibility to T1D. As a result HLA risk positive or negative is dominant over the protective or predisposing factors of CTLA-4 haplotypes.
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Genetic characteristics of latent autoimmune diabetes in adults (LADA) as a variant of type 1 diabetes mellitus
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Background and Aims: According to the recent classification of diabetes mellitus the latent autoimmune diabetes in adults (LADA) belongs to the group of Type 1 DM, however LADA comprises a heterogeneous population of diabetic patients. Whether LADA is just a variant of slowly progressing Type 1 DM or a unique identity of diabetes.
The aim of the study was to investigate the association between LADA and genes associated with high risk of development Type 1 DM: the *CTLA-4 Ala17Thr* polymorphism, microsatellite marker *D6S2414* polymorphism (located nearly HLA-DQ region) and microsatellite marker *TH01* polymorphism (located nearly IDDM2 region).
Materials and Methods: The codon 17 polymorphism in the CTLA4 gene, the microsatellite marker D6S2414 and *TH01*polymorphism were determined by PCR method in 43 LADA patients (age 48,7 ± 9,5 years, BMI 26,7 ± 4,3 kg/m, initial diagnosis of type 2 DM, but were positive for one of the three antibodies tested GAD, ICA, IAA). As control samples 31 healthy volunteers were used.
Results: LADA was positively associated with the marker *Ala17Thr* gene *CTLA-4* and the marker *D6S2414*. Distribution of alleles (not of genotypes) frequencies of marker *Ala17Thr gene CTLA-4* was differently in LADA patient and control group. The presence of the Thr17 allele was significant higher in LADA patients (62,8% and 45,2%, respectively, p<0.05 OR=2.03 (1.05–3.9)). Microsatellite marker *D6S2414*, located among genes of *HLA-*

region was positively associated with LADA. That support evidence of autoimmune origin of this form of diabetes mellitus in adult. Five alleles were determined in Marker *D6S2414*. Significant differences were received for allele 172 (1,2% in LADA and 13% in control group, p_c=0,02 OR =0,13 (0,03–0,51)). We didn't find association between LADA and microsatellite marker *TH01*, located nearly IDDM2 region, obviously IDDM2 region doesn't associate with LADA.
Conclusion: Our findings are evidence of autoimmune origin of LADA and indicate that there are marked differences in genetic background of type 1 DM and LADA.

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Susceptibility genes for type 2 diabetes

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Common variants in the *HNF-1α* gene increase susceptibility to type 2 diabetes
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Background and Aims: Mutations in Hepatocyte Nuclear Factor-1α gene (*HNF-1α*) known to cause the most common form of monogenic diabetes, Maturity Onset Diabetes of the Young type 3 (MODY3) but do common variants in the gene contribute to late onset type 2 diabetes (T2D)? *HNF-1α* is a transcription factor located on chromosome 12q24, a region to which several T2D genome wide scans have shown suggestive linkage, making it a good candidate gene for late onset T2D. To address this we studied 1) whether common variants in the *HNF-1α* gene influence transcription *in vitro* 2) searched for an association between four SNPs (rs1920792, I27L, A98V and S487N) and late onset T2D, and 3) whether these SNPs alone or in interaction with β-cell function predict subsequent T2D prospectively.
Materials and Methods: 1) Functional consequences of I27L, A98V and S487N were studied by comparing the ability of *in vitro* mutated *HNF-1α* to activate the human GLUT2 promoter or the rat albumin promoter with that of the wildtype protein. Two cell lines were used for each promotor, HeLa cells lacking endogenous *HNF-1α* and INS1 cells, expressing the corresponding rat *HNF-1α*. 2) The association study was carried out in 2000 patients with T2D the local diabetes registry and 2260 geographically matched unrelated controls from southern Sweden. 3) We performed a genotype phenotype interaction analysis in 2298 individuals from western Finland using a prospective study layout (median follow-up of 6 years).
Results: 1) *In vitro* studies, using the GLUT2 promotor in HeLa cells showed a significantly decreased transcriptional activity in carriers of the L27 ($p<0.0005$), N487 ($p<0.05$) and the combination of L27 and V98 ($p<0.0005$) alleles; in the INS1 cells this combination also showed decreased transcription ($p<0.0005$). Using the rat albumin promoter in HeLa cells both the L27 and the combination of L27 and V98 had decreased transcriptional activity ($p<0.0005$). In INS1 cells all three single variants and the combination of L27 and V98 showed decreased transcription ($p<0.0005$). 2) Genotype frequencies were in the same range as previously reported and in Hardy-Weinberg equilibrium. Three SNPs, rs1920792, I27L and S487N, were in strong linkage disequilibrium and showed nominally significant association with T2D ($p=0.03$, $p<0.0001$ and $p<0.001$). In a logistic regression analysis the homozygous L27L genotype adjusted for age, BMI and sex was associated with T2D (OR = 1.8 vs I27I; $p<0.0001$), whereas heterozygous I27L was not significantly associated with T2D (OR = 1.02, NS). 3) The L27L genotype together with the incremental 30-minute insulin during an OGTT showed a significant interaction ($p=0.03$) to predict subsequent T2D during the follow-up period.
Conclusion: The I27L polymorphism in the *HNF-1α* gene was 1) associated with impaired transcriptional activity, 2) the homozygous L/L genotype was associated with T2D and 3) interacted with the incremental 30 min insulin response to predict subsequent T2D. Although there is no obvious explanation for the discrepancy between this and our previous large report (Winckler *et al.* Diabetes in press), one possibility could be that these patients represented more severe cases ascertained from clinics in a restricted area of Southern Sweden whereas the previous report included a population based sample from both Finland and Sweden.

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The *LARS2* gene represents a novel type 2 diabetes mellitus susceptibility gene
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Background and Aims: The *LARS2* gene encodes the mitochondrial leucyl-tRNA synthetase (LeuRS). The LeuRS enzyme is responsible for the charging of mitochondrial leucyl-tRNAs with the corresponding amino acid and therefore plays an important role in mitochondrial protein synthesis. Previously we have shown that defects at the level of tRNA leucine charging are associated with Maternally Inherited Diabetes and Deafness (MIDD). Sev-

eral studies have related mitochondrial dysfunction to defects of insulin secretion, insulin sensitivity and type 2 diabetes (T2DM). We have shown that a H324Q variant in the *LARS2* gene is associated with enhanced T2DM susceptibility in four independent populations from the Netherlands and Denmark (OR 1.40 (95% CI 1.11–1.76), P=0.004, n=7836). At present it is unclear whether the H324Q is the true functional variant or is merely a reflection of linkage disequilibrium with an unknown functional variant in this locus. In the present study we have performed a detailed haplotype based analysis of the *LARS2* gene region for involvement in T2DM susceptibility.
Materials and Methods: We have performed a haplotype based association study in 360 subjects (183 T2DM and 177 controls) from the Hoorn Study, a population based study in the Netherlands. SNP genotyping was performed using PCR-RFLP and Taqman SNP genotyping assays. Haplotypes were reconstructed using Phase2.1.1 software and Powermarker was used to calculate LD values.
Results: The *LARS2* gene region spans ~160 kb on chr. 3p21.3 and consists of three haploblocks. Based on Hapmap data 11 SNPs were selected for reconstruction of haplotypes and LD pattern in 177 subjects. The individual SNP genotypes were not associated with T2DM development. Therefore we examined whether specific haplotypes were associated with T2DM susceptibility. Six SNPs were selected which can discriminate between the 8 most frequent haplotypes (≥4%). This 6-SNP combination was used to screen a larger cohort of 360 subjects from the Hoorn study (183 T2DM and 177 NGT). Haplotype analysis using these six SNPs revealed 3 additional T2DM protecting and risk haplotypes indicating that besides the H324Q variant other variants might be influencing T2DM susceptibility (all P≤0.02).
Conclusion: In conclusion, we have identified a H324Q variant in the *LARS2* gene, which enhances T2DM susceptibility. Haplotype reconstruction in DNA samples from the Hoorn study suggests that the H324Q variant is a functional variant. Furthermore it suggests the presence of additional protecting and risk haplotypes providing further evidence that the *LARS2* gene represents a novel T2DM susceptibility gene. Replication in other cohorts and functional analysis will be necessary to confirm our results. Our results further highlight the important role of mitochondria in the pathophysiology of type 2 diabetes mellitus.

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Linkage disequilibrium mapping of the type 2 diabetes susceptibility variants on chromosome 1q in European populations
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Background and Aims: A two-stage strategy starting with genome-wide linkage analysis and followed by linkage disequilibrium (LD) mapping within linked regions is a powerful approach to complex-trait gene identification. As part of the international 1q consortium, we attempted genotyping over 3000 SNPs (< 1 marker per 5 kb density) in a core 13.5Mb region of chromosome 1q (151–164.5Mb) with replicated linkage to type 2 diabetes (T2D) in ~10 genome scans.
Materials and Methods: We analysed one French, one UK and two US samples of European-descent (Amish and Utah) totaling 1239 cases (largely probands from linked families) and 1279 matched controls. Population-specific single-point and haplotype-based analyses were implemented to identify association signals likely to underlie the linkage.
Results: A Mantel-Haenszel-based meta-analysis for individual SNPs identified several such signals when examining the dominant and recessive models, including, notably, a cluster of 28 SNPs showing highly significant associations with T2D (combined odds ratios for most significantly associated SNP-specific model ranging from 1.32–1.53, p<0.001) at ~152Mb. These SNPs have high minor allele frequencies (0.17 to 0.35) and fall into a region of extended LD that includes at least twelve transcripts including the gene encoding liver pyruvate kinase, *PKLR*. Haplotypic analyses (using GENEBPM and haplotype trend regression) also provided strong evidence for association ($p < 2 \times 10^{-5}$ in UK sample and in combined European analyses).
Conclusion: These novel associations between T2D and common polymorphisms identify shared genetic susceptibility between these populations and highlight candidate genes worthy of further investigation.
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A functional variant in the human betacellulin gene promoter is associated with type 2 diabetes
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Background and Aims: Betacellulin (BTC), a member of the epidermal growth factor (EGF) family, plays an important role in regulating differentiation and growth of the pancreatic β-cells. To examine the role of polymorphisms in the promoter of human *BTC* gene, we characterized the 5'-flanking region of human *BTC* gene and screened gene polymorphisms in the promoter in type 2 diabetic patients.
Materials and Methods: To characterize the 5'-flanking region of human *BTC* gene, a series of deletions of the region were fused to a luciferase reporter gene and those constructs were transfected into βTC3 cells and their luciferase activitis were compaired. We next screened gene polymorphisms in an about 2.3 kb promoter and polymorphisms identified were genotyped in 223 type 2 diabetic patients and 254 non-diabetic subjects. We further examined the effect of polymorphism on the insulin secretion ability in type 2 diabetic patients with the plasma C-peptide response to the intravenous glucagon stimulation and the effect of polymorphisms on the promoter activity with a luciferase reporter system.
Results: The basal promoter of human *BTC* gene was located within about 200 bp region between -350 and -152 bp relative to the translation start site, and several positive and negative regulatory elements were located in the further upstream.We identified six polymorphisms (-2159A>G, -1449G>A, -1388C>T, -279C>A, -233G>C and -226A>G) in the promoter, and the G allele of -226A>G polymorphism was significantly more frequent in type 2 diabetic patients than in non-diabetic subjects (*P*=0.009). The -2159G, -1449A and -1388T alleles were in complete linkage disequilibrium with the -226G allele, but the distributions of genotypes and alleles for the -279C>A and -233G>C polymorphisms were similar between two groups.Furthermore, the plasma C-peptide response was significantly lower in the patients with the -226G allele.The promoter with the -226G allele had an about 50% decrease in the promoter activity compared with wild type, but no effects of -2159A>G, -1449G>A and -1388C>T polymorphisms on the promoter activity were observed. **Conclusion**: These results suggest that a functional -226A/G polymorphism in the human *BTC* gene promoter is associated with the development of type 2 diabetes.

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Association of the tumor necrosis factor-α (TNF-α) gene IVS1+123G/A polymorphism with type 2 diabetes
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Background and Aims: Tumor necrosis factor-α (TNF-α) is a potent cytokine that seems to have a key role in the pathogenesis of insulin resistance. Association of several promoter region polymorphisms, such as single-nucleotide polymorphisms (SNPs), -308A/G and -238A/G of the TNF-α gene with insulin resistance, obesity and type 2 diabetes (DM) have been examined in many ethnicities. However, no consistent results were observed. We here extended the association study of the TNF-α gene with DM by examining more SNPs around the region.
Materials and Methods: In addition to the SNPs, -308A/G and -238A/G, 3 SNPs upstream (namely, 2 SNPs of the TNF-β gene that locates upstream of the TNF-α gene, and 1 at position -951 (C/T) of the promoter region of the TNF-α gene) and 1 downstream (in intron 1 of the gene: IVS1+123G/A) from the SNPs -308A/G and -238A/G of the TNF-α gene were examined for the association study. A sample set (DM:72, IGT:75, NGT:227) from the cohort population of the Funagata Study was used. For some analysis, sample set was enlarged by adding 31, 77, and 244 subjects with DM, IGT, and NGT, respectively. These subjects were also from the same cohort population. Age and sex ratios were matched among the groups. Linkage disequilibrium (LD) between each pair of the SNPs was assessed by a haplotype estimation program called LD support. SNPs that showed strong LD with each other were assigned as a LD block. The haplotype frequencies were estimated using LD support. P-values for the Fisher's exact test, odds ratio (OR), and 95% CI were calculated by using a 2BY2 program. The statistical significance of the differences of the clinical trait values between the

groups was assessed by the Student's t-test. The independent association of the at-risk genotype from several clinical traits was examined by multiple logistic regression analysis.
Results: IVS1+123G/A polymorphism was associated with DM (odds ratio (OR):1.91, 95%CI:1.22–2.99. p=0.0056). An LD block from the promoter region to intron 1 of the TNF-α gene was constructed, and showed a significant difference in the haplotype distribution between the study groups (overall p=0.035). The Fisher's exact probability test showed a significant association of the haplotype 2 of the LD block (p=0.015), with an OR of 1.79 (95%CI:1.12–2.85). Multiple logistic regression analysis revealed that the at-risk genotype (AA+AG) of IVS1+123G/A polymorphism was an risk factor for DM, independently from systolic blood pressure, west circumference, TG, HDL cholesterol and BMI, with OR of 1.51 (95%CI:1.09–2.09). Subjects with the at-risk genotype of IVS1+123G/A polymorphism was not insulin resistant (HOMA-R:1.10 ± 0.85 vs. 1.02 ± 0.65, P=0.228), but has more body fat (percent body fat: 25.9 ± 7.1 vs. 24.4 ± 7.5, p=0.046) compared to the others.
Conclusion: TNF-α gene IVS1+123G/A polymorphism was associated with type 2 diabetes in a Japanese population.

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An insertion/deletion polymorphism in the Alpha 2b-adrenoceptor gene is associated with age at onset of type 2 diabetes mellitus
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Background and aims: The human Alpha 2B adrenoceptor (Alpha 2B-AR) mediates a variety of functions, including insulin secretion. An insertion/deletion (I/D) polymorphism of the Alpha 2B-AR gene located on chromosome 2 has recently been described. There is evidence that presence of the deletion form (D allele) of this gene may be connected with diminished insulin secretion and progression to diabetes in patients with impaired glucose tolerance. Therefore, the aim of the present study was to examine if there is a difference in the D allele frequency of Alpha 2B-AR gene between type 2 diabetic patients and controls, as well as to ascertain whether the D allele is associated with earlier onset of type 2 diabetes.
Materials and methods: This study included 403 subjects divided into two groups. Group A comprised 199 type 2 diabetic patients (96 men, mean age 66.55 ± 9.28 years, mean diabetes duration 11.94 ± 8.05 years), while group B comprised 204 age-and-sex-matched healthy volunteers (101 men, mean age 66.04 ± 8.63 years). Genomic DNA was extracted from whole blood. Genotyping of I/D polymorphism was performed by Polymerase Chain Reaction (PCR). The following primers were used: Forward: 5' -accagatctcaaaaagaaggtct-3' and Reverse: 5' -catgatggtgaagataagcctcaca-3'. The I and D alleles appeared as 112-base pair and 103-base pair amplicons respectively.
Results: In group A frequencies of genotypes I/I, I/D and D/D were 61.81%, 32.16% and 6.03% respectively, while the same frequencies were 65.69%, 30.39% and 3.92% in group B. No significant difference in the D allele frequency was observed between the two groups (group A: 22.11% and group B: 19.12%, Yates-corrected Chi-square=0.681, p=0.409). In group A, age at onset of diabetes was significantly (t=5.668, df=197, p<0.001) lower in patients carrying the D allele (N=74, mean age at onset of diabetes 51.49 ± 8.62 years) as compared to those without the D allele (N=125, mean age at onset of diabetes 59.27 ± 9.78 years).
Conclusions: No difference appears to exist in the allele frequency of Alpha 2B-AR gene between type 2 diabetic patients and age-and-sex-matched healthy volunteers. Among type 2 diabetic patients, however, presence of the D allele is associated with significantly younger age at onset of diabetes. This result indicates that the D allele may be implicated in impaired glucose homeostasis contributing to earlier manifestation of type 2 diabetes in predisposed subjects.

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The G4863A polymorphism in the coding region of the GRB10 gene is associated with reduced risk of type 2 diabetes in Caucasians from Italy
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Background: The Insulin Resistance Syndrome (IRS) also known as the Metabolic Syndrome plays a crucial role in the pathogenesis of type 2 diabetes (T2D) and cardiovascular disease. It recognizes a genetic background which is mostly unknown. Genes encoding for proteins affecting Insulin Receptor Tyrosine Kinase activity (IR-TK) and downstream signaling have been reported to modulate whole body insulin sensitivity. To study genetic variations in these genes would help understand the molecular mechanisms of the IRS and T2D. Several proteins inhibiting the IR-TK and insulin action have been described. Among these is a docking protein Grb10. The aim of this study was to investigate whether SNPs in the coding regions of Grb10 genes may contribute to the IRS and T2D.
Subjects recruitment Both cases and controls were of Caucasian origin and resident of the same region (i.e. Gargano and surrounding areas, East Coast of Central Italy). Cases were patients with T2D who met the following criteria: i) diabetes diagnosed after 30 yr; ii) insulin treatment not required for at least 2 yr after diabetes diagnosis; iii) absence of clinically evident autoimmune disease.
The control group consisted in unrelated subjects with the same range of age (35–76 yrs) as cases who were recruited in the same area as part of the Telethon project E1239, 2000. Selection criteria were as follows: fasting plasma glucose less than 6.1 mmol/l, no medications known to affect glucose and lipid metabolism and absence of systemic diseases.
GRB10 Polymorphic Screening: By screening 100 chromosomes of our populations for the entire coding region of Grb10, 7 SNPs were identified. The allelic structure and linkage disequilibrium between SNPs (according to http://www.hapmap.org/index.html.en) indicated the presence of two haplotype blocks. Three out of the seven identified SNPs (G461A, T36487C, G41221A) lie in a block of 4 kb at the 5'region of the gene. A second block starts with SNP G4863A in the coding region, ending with G4004A SNP in the 3' untranslated region. The linkage disequilibrium of each haplotype block remains high from the 5'upstream SNP (G461A and G4863A respectively) to the more distal SNP (G41221A, G4004A respectively). Due to their high r2 values, as measured in comparison to the other SNPs in the block, we believe that the G461A and the G4863A are the most informative SNPs of each block. Therefore, the G461A and G4863A SNPs were tested for association with T2D.
Results: The proportion of controls and T2D patients carrying the G4863A SNP was significantly different while it was similar for the G461A SNP. In fact, the risk of being affected by T2D was greatly reduced (approximately 75–80% reduction) for subjects carrying the 4863A allele (OR=0.235, C.I. 95% 0.17–0.32, p<0.001); this significant reduction persisted also when age, gender and BMI were considered into the model (OR 0:25 CI 95% 0.16–0.40, p>0.001).
A putative biological significance of the G4863A is suggested by ESEfinder analysis (http://exon.cshl.edu/ESE/) which identifies a putative exonic splicing enhancer that might be disrupted by the 863A variant.
Functional analysis of the G4863A SNP, by the minigene approach, is currently under investigation in our laboratory.
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Genetic and non-genetic factors regulate expression of genes involved in oxidative phosphorylation in human skeletal muscle
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Background and Aims: A set of genes involved in oxidative phosphorylation (OXPHOS genes) show reduced expression in muscle of patients with type 2 diabetes, but it is unknown whether this is an inherited or acquired defect. Our aim was therefore to study the influence of genetic and non-genetic factors on the expression of 4 OXPHOS genes, NDUFB6 (complex 1), UQCRB (complex 3), COX7A1 (complex 4) and ATP5O (complex 5) in human muscle biopsies.
Materials and Methods: Skeletal muscle biopsies were taken from young (n=86, age 28 ± 0.2 years) and elderly (n=68, age 62.4 ± 0.2 years) monozygotic (MZ) and dizygotic (DZ) healthy twins before and after a hyperinsulinemic euglycemic clamp.
Results: The mRNA levels of NDUFB6, UQCRB, COX7A1 and ATP5O were significantly reduced (15%-40% reductions; P<0.002) in muscle biopsies from elderly compared with young twins. Using a classical twin approach, we could study heritability of muscle OXPHOS expression. In young twins, the intraclass correlations were significant different between the MZ and DZ twins for insulin-stimulated mRNA levels of NDUFB6 (r=0.55 vs. r=0.098; P<0.05), COX7A1 (r=0.70 vs. r=0.32; P<0.0001) and ATP5O (r=0.61 vs. r=0.26; P<0.05), demonstrating genetic components. In the elderly twins, the intraclass correlations were different between the MZ and the DZ twins for basal mRNA levels of NDUFB6 (r=0.60 vs. r=0.13; P<0.05), COX7A1 (r=0.69 vs. r=0.26; P<0.005) and ATP5O (r=0.74 vs. r= –0.064; P<0.0001) as well as for insulin-stimulated NDUFB6 expression (r=0.64 vs. r=-0.02; P<0.005). By the use of a generalised estimating equa-

tion model we tested whether the following factors: basal and insulin-stimulated muscle PGC-1α and PGC-1β mRNA levels, the *PGC-1α Gly482Ser* polymorphism, birthweight, age, sex, percentage body fat, VO2 max, and the interactions between: sex and percentage body fat, sex and VO2 max, age and percentage body fat, age and VO2 max, and age and *PGC-1α Gly482Ser* influence the mRNA level of NDUFB6, UQCRB, COX7A1 and ATP5O in muscle. The mRNA level of NDUFB6 was significantly influenced by PGC-1α expression, sex, percentage body fat and the interaction between age and *PGC-1α Gly482Ser.* UQCRB expression was influenced by PGC-1α, PGC-1β, percentage body fat, sex, birthweight and the interaction between age and *PGC-1α Gly482Ser.* COX7A1 expression was influenced by PGC-1α, PGC-1β, VO2 max, age, sex and percentage body fat. ATP5O expression was influenced by PGC-1α, sex, VO2 max, and age. Finally, a SNP (A/G) was identified in NDUFB6, influencing both gene expression and metabolism in muscle. Elderly carriers of the G/G genotype had reduced NDUFB6 levels compared with carriers of A/A and A/G genotypes (G/G 0.24 ± 0.03 vs. A/G 0.33 ± 0.02 and A/A 0.36 ± 0.02; P<0.05). Also, elderly carriers of a G-allele had reduced insulin stimulated glucose disposal (G/G 264 ± 28, A/G 233 ± 14 vs. A/A 294 ± 13; P<0.05), VO2 max (G/G 24 ± 2, A/G 24 ± 1 vs. A/A 29 ± 1; P<0.05), nonoxidative glucose metabolism (G/G 146 ± 27, A/G 128 ± 13 vs. A/A 187 ± 13; P<0.05) and increased percentage body fat (G/G 30 ± 3, A/G 29 ± 1 vs. A/A 26 ± 1; P<0.05) compared with the A/A group.
Conclusion: We show that muscle OXPHOS expression is influenced by both genetic and non-genetic factors (e. g. age), thereby demonstrating how genes and environment may interact to influence age-dependent susceptibility to type 2 diabetes.
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PS 12
Genetic determinants of cardiovascular risk in type 2 diabetes

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Adiponectin gene polymorphisms – a genetic link between predisposition to type 2 diabetes and coronary artery disease
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Background and Aims: Adiponectin - a newly discovered cytokine secreted by adipose tissue was recently found to play an important role in the pathogenesis of insulin resistance and the development of atherosclerosis. The aim of our study was to evaluate the relationships between the adiponectin gene polymorphisms and coronary artery disease (CAD) in 657 patients with (n=369) and without (n=288) type 2 diabetes, who had undergone coronary angiography.
Materials and Methods: CAD was defined as more than 50% reduction in diameter of at least one major vessel and confirmed in 410 patients, while 247 subjects had coronary vessels without any or with less than 50% narrowings. In all subjects we determined the presence of three SNPs (-11426A/G, -11391G/A, -11377C/G) in the promotor region, SNP+45T/G in exon 2, SNP+276G/T in intron 2 and rare mutations in exon 3 (G84R, G90S, Y11H, I164T) by direct sequencing of the appropriate fragments of adiponectine gene. Moreover in 460 randomly chosen participants of the study we determined adiponectin levels in the peripheral blood.
Results: In our study the frequency of -11391A allele was lower in patients with CAD in comparison to subjects without significant stenosis in coronary vessels (5.1% vs. 10.3%, p=0.0003) and in type 2 diabetes in comparison to persons without glucose metabolism disturbances (5,3% vs. 9,4%, p=0.006). In addition, our results have confirmed that plasma adiponectin level is modulated by variants of APM1gene (-11391G/A, +276G/T, Y111H).
Conclusions: To our knowledge this is the first study which shows that SNP -11391G/A is associated not only with and type 2 diabetes mellitus (OR=0.54, p<0,005), but also with the lower risk of coronary artery disease (OR = 0.46, p<0,001). Our results suggest that SNP-11391G/A of adiponectin gene may provide a genetic link between predisposition to type 2 diabetes and CAD.
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Leucine 7 to proline polymorphism of prepro neuropeptide Y: increased risk for cardiovascular disease in obese subjects with abnormal glucose regulation –The Hoorn Study
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Background and Aims: NeuropeptideY (NPY) is a sympathetic transmitter both in the central and peripheral nervous system. A common Leucine 7 to Proline 7 (Leu7Pro) polymorphism of preproNPY is associated with altered sympathetic transmitter and hormone release and altered autonomic cardiovascular function in healthy young subjects and with increased risk for complications in diabetic subjects. We investigated the impact of the Leu7Pro polymorphism on obesity, abnormal glucose regulation and cardiovascular disease (CVD) in elderly population.
Materials and Methods: The subjects were participants of a population-based cohort study, the Hoorn Study. Subjects, 128 with BMI ≥28 (obese) and 312 with BMI <28 (non-obese), who attended the baseline (1989) and follow-up medical examination (1996–1998), were genotyped for the Leu7Pro polymorphism of preproNPY. Subjects were followed with respect to morbidity and mortality. CVD was considered as non-fatal or fatal cardiovascular event or sudden death by the year 2000. Subjects were categorized according to genotype, abnormal glucose regulation (AGR: IGT, IFG or diabetes) and obesity status at the second examination. The risk for CVD

was evaluated in the 8 groups separately by using Cox survival analysis. The genotype-obesity interaction effect on developing AGR was evaluated by using logistic regression model.
Results: There was a significant genotype-obesity interaction effect on development of AGR (p = 0.037) (Table). All the obese subjects with the Leu7Pro polymorphism had AGR compared to 63.4% of obese subjects without this polymorphism while of the non-obese subjects with the Leu7Pro polymorphism 20.0% had AGR compared to 40.8% of those without the polymorphism. 83.3% of obese subjects with the Leu7Pro polymorphism had CVD compared to 38.2% of those without the polymorphism. The incidence of CVD in non-obese subjects was similar in both genotypes (20.0% and 25.6%, respectively, p = 0.627). The Leu7Pro polymorphism was associated with significantly increased risk of incident CVD in obese subjects with AGR compared to the obese subjects with AGR and without the polymorphism (Hazard ratio (HR) 6.391, CI 95% for HR 2.35–27.30, adjusted for gender, age, OGTT status and BMI at baseline).
Table

	HR	95.0% CI for HR Lower	Upper
Leu7Pro	0.46	0.16	1.32
Obesity	2.71*	1.69	4.34
Leu7Pro-by-obesity	12.17**	1.17	126.77

Independent associations of the Leu7Pro polymorphism, obesity and the Leu7Pro polymorphism-obesity interaction with developing abnormal glucose regulation
CI, Confidence interval; * p <0.001, ** p <0.05

Conclusion: There was a significant interaction between the Leu7Pro polymorphism and obesity. The Leu7Pro polymorphism was associated with increased risk for AGR in obese subjects and for CVD in obese subjects with AGR. The mechanisms remain still unclear and require further investigations.

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T-344C polymorphism in the aldosterone synthase (CYP11B2) gene (CYP11B2) and blood pressure in type 2 diabetics (DT2) with high vascular risk. (DIABHYCAR Study)
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Background and Aims: The renin-angiotensin-aldosterone-system regulates the blood pressure. The aldosterone synthase gene, CYP11B2, encodes for a cytochrome P450 enzyme, involved in the terminal steps of aldosterone synthesis in the zona glomerulosa cells of human adrenal glands and its expression is regulated by angiotensin II and potassium. A functional variation (T-344C) in the promoter region of the CYP11B2 gene has been associated with hypertension. We aimed to test the association between this polymorphism and blood pressure and urinary albumin excretion (UAE) in a large cohort of high vascular risk type 2 diabetics (DT2) followed prospectively.
Materials and Methods: Subjects took part in DIABHYCAR (DIABetes HYperetension CArdiovasculaire Ramipril) a randomized low-dose ramipril placebo-controlled cardiovascular prevention trial. The 4912 subjects were micro- or macroalbuminuric (UAE >20 mg/l in 2 samples) type 2 diabetic subjects who were 50-year-old or more, used oral anti-diabetic drugs and had a creatininaemia <150 mM. Genotyping for the T-344C in CYP11B2 gene was done by a high-throughput technique: DNA amplification by Polymerase Chain Reaction was followed by an allelic-specific hybridization (Molecular Beacon®, MWG). ANOVA was used to test the effect of the genotypes on blood pressure (≥140/90 mmHg and/or antihypertensive treatment) and UAE, with adjustment for age, body mass index and duration of diabetes in the whole population and after stratification according to gender status.
Results: The genetic study was performed on the 3105 French out of the 4912 participants to the DIABHYCAR Study. Allelic frequencies were 54 and 46% for T and C respectively. The genotypes and hypertension at inclusion were associated (1752 hypertensives: TT = 31,5%, TC = 48,6% and CC = 19,9% and 1363 non hypertensives: TT = 27,7%, TC = 50,2% and CC = 22,1%; p = 0.016 after adjustment). In the whole population, the association between the systolic blood pressure (SBP) and this variant failed to be significant (p = 0,066 after adjustment). In women (n = 834), the polymorphism was associated with SBP: TT: 144,3 ± 0,9 (n = 256), TC: 147,5 ± 0,7 (n = 418), and CC: 145,6 ± 1,1 mmHg (n = 160); p = 0,009 after adjustment. No significant association was found between the genotype and UAE in the whole population or after stratification by gender.
Conclusion: In DT2 participant with high vascular risk, we detected an association between a functional single nucleotide polymorphism in CYP11B2 gene and hypertension, particularly in women. This study provides a possible genetic link between blood pressure homeostasis and aldosterone synthase in DT2.

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The +276G>T adiponectin gene SNP is associated with cardiovascular disease in patients with type 2 diabetes mellitus
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Background and Aims: Adiponectin is an adipocyte specific plasma protein that plays a role in the regulation of insulin action and energy homeostasis. Recently anti-inflammatory and anti-atherogenic properties have been also been described. Low circulating serum adiponectin levels are associated with obesity, insulin resistance, type 2 diabetes (T2DM) and coronary artery disease (CAD). A focus associated with CAD has been identified in a region encompassing the adiponectin gene but to date the evidence that specific gene variants are associated with cardiovascular disease have been contradictory. We aimed to determine if variation in a single nucleotide polymorphism at position +276 in the adiponectin gene is a risk factor for CAD in patients with T2DM.
Materials and Methods: The University College, Diabetes and Cardiovascular disease study (UDACS) is a cross sectional study designed to examine the effect of differences in inflammatory genes on cardiovascular risk. Consecutive attenders at the diabetes clinic at UCL hospitals were recruited in 2001–2002. CAD was defined as documented myocardial infarction, revascularisation, angina of any type or positive cardiac investigation including exercise test, nuclear medicine or angiography. Genotype was determined by PCR amplification and RFLP analysis.
Results: For this study we examined only male subjects with T2DM (N=483). Genotype was determined in 478 patients (99%). The variant T allele was more frequent in the subjects with CAD (Frequency[95%Confidence Interval]; 0.32[0.26–0.37] vs 0.26[0.22-0.29]; p=0.04). There was no difference in age, lipids, blood pressure, HbA1c, duration of diabetes or smoking status between the two groups. For homozygotes (N=282) the relative risk of CAD for the variant alleles, TT v GG was 2.41 (1.13–5.15:p=0.02).
Conclusion: The variant +276T allele of the adiponectin gene is associated with CAD in diabetes. Such an association has also been found in a group of Italian men with diabetes. Further study of the relationship between this gene variant and phenotype is required as this variant is in a biological non- functional intron. In any event the +276G>T adiponectin gene variant may act as a genetic marker to identify patients with T2DM patients who are at increased risk of CAD and may thus benefit from a more aggressive risk prevention strategy.
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Genetic and demographic processes in modern populations: the metabolic syndrome base clinical components manifestation
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Background and Aims: Modern urbanized populations are characterized by high crossbreeding degree, intensive migration processes and coadaptive gene complexes destruction. These processes affect the expressiveness and frequency of heredity pathologies. But the influence of population processes on the metabolic syndrome (MS) base clinical components – type 2 diabetes mellitus (DM), essential hypertension (EH) and abdominal obesity – is still unknown. The aim of the study was to assay the impact of

the crossbreeding degree (CD), migration distance (MD) and parents marriage distance (PMD) of the proband on the manifestation of the MS base clinical components.
Materials and Methods: 330 probands with EH (BMI 28.12 ± 0.76 kg/m^2), their 1st and 2nd degree relatives (3890 subjects), 229 persons with abdominal obesity (BMI 35.33 ± 0.98 kg/m^2), their 1st and 2nd degree relatives (2015 ones) and 472 Type 2 DM patients (BMI 29.86 ± 0.83 kg/m^2) and 5626 1st and 2nd degree relatives were enrolled. CD (parents and grandparents birthplace is the same rural area - 1CD, parents belong to the same ethnically group and have different birthplaces – 2CD, parents are ethnically similar – 3CD and parents are ethnically remote - 4CD), MD and PMD of proband were analysed.
Results: It has been shown, that unlike obesity type 2 DM and EH family accumulation is correlate with proband CD ($r_{obesity}$= 0.022, χ^2 = 3.02, P>0.05; $r_{DM\,2}$ = 0.041, χ^2 = 16.31, P < 0.001; r_{EH} = 0.042, χ^2= 13.65, P<0.001). The proband CG increase was accompanied by the type 2 DM and EH family accumulation growth (1CD – 4.27%; 2CD – 7.23%; 3CD – 6.40%; 4CD – 6.99% for type 2 DM and 1CD – 13.09%; 2CD – 15.44%; 3CD – 16.46%; 4CD – 15.86% for EH, respectively). Proband MD affects the relative stability to obesity (r= 0.05, χ^2 = 10.08, P<0.05). MD influences also on the stability to EH (r_{1CD}=0.087, χ^2 =13.39, P<0.01; r_{2CD}=0.045, χ^2 =5.77, P>0.05; r_{3CD}=0.089, χ^2 =20.54, P<0.01; r_{4CD}=0.293, χ^2 =48.33, P<0.001). MD for type 2 DM has impact on the disease manifestation only in the probands with 2CD (r = 0.044; χ^2 = 9.504, P < 0.05). Proband PMD has not influence on the obesity and type 2 DM manifestation - family accumulation of obesity and type 2 DM does not correlate with PMD ($r_{obesity}$=0.039; χ^2 = 5.61, P>0.05; for type 2 DM r_{2CD}=0.042, χ^2 =7.77, P>0.05; r_{3CD}=0.065, χ^2 =8.04, P>0.05; r_{4CD}=0.078, χ^2 =7.07, P>0.05). In EH proband PMD correlates with family accumulation (r_{2CD}=0.084, χ^2 = 18.32, P<0.01; r_{3CD}=0.107, χ^2 = 20.54, P<0.001; r_{4CD}=0.055, χ^2 = 15.04, P<0.01).
Conclusion: Genetic and demographic processes are different influence on the MS base clinical components manifestation: the obesity manifestation is only proband MD affected, on type 2 DM manifestation – proband CD, the EH manifestation – MD, PMD and CD of proband.

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CCAAT enhancer binding proteins as candidate transcription factors in the regulation of features of the insulin resistance syndrome
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Background and Aims: We hypothesised that common variants in the genes encoding members of the CCAAT enhancer binding protein (C/EBP) family of transcription factors may explain the clustering of haemostatic and metabolic proteins in type 2 diabetes and cardiovascular disease. The aim of this study was to screen the genes encoding members of the C/EBP family of transcription factors for common polymorphic variants and to determine their association with haemostatic factors and features of the insulin resistance syndrome.
Materials and Methods: We screened the entire coding region and 3 kb of 5' gene regulatory region of the genes encoding CEBPA, CEBPB and CEBPD for novel polymorphisms by dHPLC using the Transgenomic WAVE. Identified polymorphisms were then genotyped in 508 subjects from 89 families in the Leeds Family Study and data were analysed using SPSS v12 and SOLAR to determine the contribution of polymorphic variants to the heritability of haemostatic and metabolic variables.
Results: We identified 13 common polymorphic variants in CEBPA, 10 in CEBPB and 1 in CEBPD. In univariate analyses we identified significant associations of C/EBP polymorphisms with several haemostatic and metabolic variables, including FXIII, adiponectin, leptin and WHR, although the contribution of individual polymorphisms to variance in traits was only between 1–2% in each case and different polymorphisms were associated with different variables. In multivariate analyses including biochemical and demographic variables, the associations of a number of the polymorphisms in CEBPA and CEBPB with haemostatic and metabolic variables remained significant, including FXIII and adiponectin.
Conclusion: These data suggest that common polymorphic variants in the C/EBP family of transcription factors may result in modulation of the transcriptional regulation of target genes. Modest influences on a number of haemostatic and metabolic factors may translate into a larger influence on overall risk for type 2 diabetes and cardiovascular disease, which will be the subject of follow-up studies.
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Calpain-5 gene variants are associated with features of the metabolic syndrome
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Background and Aims: Genes implicated in common complex disorders such as type 2 diabetes mellitus (T2DM) or cardiovascular disease remain elusive because the genetic contributions of the individual loci are small, influencing but not determining overall disease susceptibility. Cysteine protease CAPN10 has been associated with T2DM and other components of the metabolic syndrome (MS). The aim of this study was to investigate the relationships between the calpain 5 gene (CAPN5), a CAPN10 homologue, T2DM and the MS (ATPIII criteria).
Materials and Methods: We studied 606 non-related Caucasian subjects aged 35–76 years, randomly identified from a cross-sectional population-based epidemiological survey in the province of Segovia in Central Spain (Castilla-León). Phenotype measurements: BMI, waist circumference, systolic (SBP) and diastolic blood pressures (DBP), fasting glucose, OGTT, fasting insulin, insulin resistance (HOMA-IR), lipid profile. Genetic variants were screened using either pyrosequencing technique in a PSQ^{tm} 96 (Pyrosequencing AB) or spectrofluorometric methods in a LightCycler system (Roche). Haplotype analysis was performed using the THESIAS software (http://www.genecanvas.org). This method allows one to estimate haplotype frequencies and haplotype effects by comparison to a reference (the intercept) taken here as the most frequent one. Haplotypes effects are expressed as increases/decreases of the phenotypic mean with respect to the intercept's one.
Results: The following variants were found in the 5' region: g.86A>G, and g.344 G>A. Two other variants were found in the intron 3: c.1320C>T and c.1469 G>A. Frequencies were 0.74 for A allele in Nt g.86A>G, 0.57 for G allele in Nt g.344 G>A, 0.94 for C allele in Nt c.1320 C>T and 0.80 for G allele in Nt c.1469. Genotypic analysis of all SNPs with phenotype measurements by Kruskal-Wallis ANOVA test has only identified lower DBP values for individuals homozygotes for wild type allele (G, allele 1) at Nt g.344 G>A (p=0.017).
Haplotype analysis showed that the highest global haplotypic effect was for DBP (χ^2=17.6 with 5 d.f, p=0.0035) followed by fasting total cholesterol (χ^2=13.05 with 5 d.f, p=0.023) and LDL-cholesterol (χ^2=11.91 with 5 d.f, p=0.03). BMI and HDL-cholesterol showed a trend toward being associated at this level (p=0.056 and 0.07, respectively).
We found a protective effect of CAPN5 haplotype AGCA from abdominal obesity (p=0.011) having this haplotype 12.5% lower waist circumference mean that the one with the most frequent haplotype (AACG). Associations on DBP were also found with haplotype AGCA, having 14.2% lower DBP than the intercept ($p < 10^{-5}$), and with haplotype GGCG (6.4% under intercept phenotypic mean, p=0.002). Moreover the AACA haplotype was associated with increased risk for the metabolic syndrome (OR:1.68 CI95% 1.02–2.75, p=0.039) and show trend of being associated with higher BMI values (p=0.08). Total and LDL-cholesterol levels were higher in individuals bearing the GGCA haplotype (+28.9% p=7x10-4, and +36.8% p=0.003 respectively). Increased HDL-cholesterol levels were associated with the AGCA haplotype (+28.8% p=0.001).
Conclusion: Our results indicate that the CAPN5 gene may be a gene influencing several components of the metabolic syndrome such as diastolic blood pressure and lipid profile.
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The GNAI2 promoter -318C>G polymorphism decreases transcriptional activity and is associated with hypertension in Caucasians from Italy
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Background and Aims: Inhibiting G alfa subunit 2 protein (G_{i2alfa}) has been reported to be involved in the pathogenesis of essential hypertension and/or insulin resistance (IR). Aim of this study was to determine whether variations in GNAI2 gene, encoding for G_{i2alfa} protein, contribute to hypertension and/or IR.
Materials and Methods: To this purpose, all the exons, 1500 bp of the promoter region and the 3' UTR of the GNAI2 gene were resequenced in 48

individuals. Seven single nucleotide polymorphisms (SNPs) at the GNAI2 locus were identified. Due to the low allelic frequency (AF) (i.e. < 5%) and nature (i.e. synonimous or intronic) of the other SNPs, only the -318C>G (6% AF) in the promoter region was studied for association with blood pressure and other features related to IR, including BMI, waist circumference, fasting serum insulin, glucose and lipids.
Results: This SNP lies in a 14 Kb LD block covering the 5' half of the gene and is informative of all this area. The -318C>G SNP was typed in 655 non-diabetic Caucasians (406F/249M, age 36.8 ± 11.7, BMI 25.5 ± 4.5) from Centre East Coast of Italy (employees of our Institution not taking medications known to interfere with blood pressure and IR-related features) and found to be significantly associated with higher systolic blood pressure (117.8 ± 16 mmHg vs 113.6 ± 12.6 in C/G and C/C subjects, respectively; p=0.016, adjusted for age, gender and BMI), but not with other IR-related features. C/G carriers had a significantly increased risk to be hypertensive (SBP ≥130 mmHg and/or DBP≥85 mmHg) than C/C individuals (OR: 2.1, 95% C.I.=1.1–4.2, p=0.035). Transfections of HEK293 cells with luciferase reporter constructs of 450 bp GNAI2 promoter sequence containing either the -318 C or the G allele, showed that the G allele had 2.5 fold reduced transcriptional activity, as compared to the C allele (n=6 experiments, p<0.05). Electrophoretic mobility shift assays showed that nuclear proteins from HeLa cells exhibited a lower binding affinity to the probe harboring the G allele as compared to that harboring the C allele and that transcription factors Yin Yang 1 and Sp1 differently bound to C and G allele.
Conclusion: In conclusion, we have conducted the first genetic study of GNAI2 (the gene encoding for G_{i2alfa}) in relation to hypertension and other metabolic phenotypes related to IR. The -318C>G is associated with higher blood pressure and essential hypertension. Functional studies, indicating different transcriptional activities of the 2 alleles, provide a functional basis for this association.
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CETP polymorphisms and glucose tolerance status. The Hoorn Study
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Background and Aims: We have studied the association between glucose tolerance status and variation at the gene locus encoding Cholesterol Ester Transfer Protein (CETP). This lipid transfer protein plays a key role in human lipid metabolism. The TaqIB gene polymorphism is closely linked with the C-629A gene polymorphism and is associated with plasma CETP concentration, and with high-density lipoprotein cholesterol (HDL-C).
Materials and Methods: 758 subjects, selected for glucose tolerance status, participated in the 2000–2001 follow-up examination of the Hoorn Study. Fasting and post-load glucose levels, serum lipids and CETP concentration were determined and DNA was extracted from blood cells for genotyping.
Results: No differences in CETP concentration were found between subjects with normal glucose metabolism (NGM), impaired glucose metabolism (IGM) and diabetes mellitus (DM) (Table). Subjects with IGM were older than subjects with NGM. The AA genotype (p<0.05) and the B2B2 genotype (p=0.05) were most prevalent in subjects with IGM (Table). As expected, subjects with AA and with B2B2 genotype had the highest HDL-C (p=0.04, p=0.02) and the lowest CETP concentration (p<0.01 for both) (data not shown).
Conclusion: Since the B2 and A alleles were more common in subjects with IGM then in subjects with NGM or DM, and are associated with an anti-atherogenic lipid profile, it may be speculated that these genotypes might be protective against development of diabetes. Prospective studies are needed to verify these findings.

Characteristics of the study population in categories of glucose tolerance status (n=758)

	NGM	IGM	DM
n	279	176	303
Age (years)	68.8 (6.1)	70.2 (6.4)*p<0.05	68.6 (7.9)
Gender (%male)	48.4	49.4	51.5
CETP (mg/l)	1.88 (0.56)	1.93 (0.58)	1.86 (0.63)
-629 CC (%)	32.0	26.7	30.6
-629 CA (%)	47.8	43.5	47.8
-629 AA (%)	20.2	29.8 *p<0.05	21.6
TaqIB B1B1 (%)	36.3	30.2	33.7
TaqIB B1B2(%)	47.3	45.7	50.2
TaqIB B2B2 (%)	16.4	24.1 *p=0.05	16.1

Values presented as means (standard deviation). t-test was used for comparison with NGM subjects.

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Identifying people at risk of type 2 diabetes

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Cutoff value(s) for fasting plasma glucose: relation with IFG prevalence, risk of diabetes and cardiovascular disease

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Background and Aims: The cost/benefit balance of lowering the diagnostic threshold for impaired fasting glucose (IFG) from 6.1 to 5.6 mmol/l is currently debated. We evaluate 1) the impact of the new diagnostic criteria on prevalence of IFG across sex, age, and BMI strata; 2) the risk of diabetes and CVD among subjects with varying values of fasting plasma glucose (FPG).
Materials and Methods: 40419 non diabetic people, aged 30–70 years, were studied. Measurements include BMI, waist circumference, blood pressure, FPG, lipids. Normoglycemia is defined as FPG <5.6 mmol/l. Participants were stratified according to FPG (mmol/l): <4.9; 4.9–5.2; 5.3–5 (i.e.tertiles 1–3 of the normoglycemic range); 5.6–6.1 (i.e. additional IFG cases diagnosed with the 2003 criteria), 6.1–6.9 (IFG by 1997 criteria).
Results: by lowering the IFG diagnostic threshold the prevalence of this condition increases from 12.3% to 42.9% (i.e by 300%). The observed increases vary with age, gender and BMI ranging from 400% in normal-weight females to 130% in males with BMI >= 35 kg/m^2). Prevalence of major risk factors for diabetes or CVD measured in the study progressively increase with FPG values with a significant linear trend. OR for high CVD risk – i.e. coexistence of three or more among LDL cholesterol>=3.4, triglycerides >=1.69, HDL cholesterol <1.04 mmol/l, blood pressure>= 140/90 mmHg - were 1.12; 1.09; 1.10 and 1.15, respectively for those with FPG 4.9–5.2; 5.3–5; 5.6–6.1 and 6.1–6.9 vs FPG <4.9 (all statistically significant). Risk of diabetes (i.e. coexistence of three or more among age >=55 years, BMI>=30 kg/m^2, waist >=88(F) >=102(M), use of antihypertensive medication), was significantly increased only for the groups with FPG 5.6–6.1 and 6.1–6.9, OR vs FPG <4.9 are respectively 1.21 (1.14–1.30) and 1. 35 (1.13–1.17)
Conclusion: with the new criteria for IFG the largest increase in prevalence of the condition is observed in younger and non obese people. Risk of CVD increases gradually and significantly with FPG values, if a threshold exists this may be lower than the value recommended for diagnosis of IFG. For risk of diabetes a threshold effect is detected at a value near to the new recommended cut-off for IFG.

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The reduction of cut-point of impaired fasting glucose on the risk of diabetes and cardiocerebrovascular events: a prospective study in Shanghai communities population

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Background and Aims: To compare the relationship between impaired fasting glucose (IFG), which was diagnosed according to the WHO (1999) criteria or the recommendation that American Diabetes Association (ADA) suggested in 2003, and the risk of developing diabetes and cardiovascular and cerebrovascular (CVD) events, as well as to investigate the feasibility of lowering the cut-point of IFG from 6.1 mmol/l to 5.6 mmol/L in Chinese.
Materials and Methods: From 1998 to 2001, we conducted a cross-sectional survey on metabolic syndrome and its components in Shanghai residents aged over 20 years in Shanghai Huayang and Caoyang community. From 2002 to 2004, we conducted a follow-up survey on these previously examined subjects. BMI, waist circumference, waist to hip ratio and blood pressure were measured, and fasting insulin and lipid profile were detected, as well as 75-gram glucose tolerance test (OGTT) were performed in those subjects without validated diabetes history. In addition, the questionnaires on the incidence of CVD events were completed.
Results: 1. Of 4957 subjects without diabetes in the baseline survey, 2595 of them underwent the OGTT test. The 3-year cumulative incidence of diabetes was 4.39% in these 2595 individuals, the annual incidence was 1.46%. 2. In those subjects with normal glucose tolerance and impaired glucose regulation according to WHO criteria, the annual incidence of diabetes was 0.80% and 5.83% respectively. 3. Among the subjects with FPG 5.6–6.0 mmol/L and 2 h-PG<7.8 mmol/L, and those with FPG 6.1–6.9 mmol/L and 2 h-PG<7.8 mmol/L, the risk of diabetes increased 4.44 times and 20.40 times (P all <0.001) respectively. 4. Of 5242 subjects without CVD events in the baseline survey, 2831 of them completed the questionnaires on the incidence of CVD events in this follow-up. Among the subjects with FPG <5.6 mmol/L and 2 h-PG 7.8–11.0 mmol/L, and those with FPG 5.6–6.0 mmol/L and 2 h-PG 7.8–11.0 mmol/L, the risk of CVD events increased 2.11 and 2.65 times (P all <0.01) respectively.
Conclusion: The risk of developing diabetes in those subjects with FPG of 5.6–6.0 mmol/L markedly increased. The lower cut-point defining IFG should be reduced from 6.1 mmol/L to 5.6 mmol/L, which will be of advantage to the prevention of diabetes. The predictive value of the reduction of lower cut-point defining IFG on CVD event was not observed in this study.

Risk of developing diabetes and CVD events in different FPG and 2 h-PG levels

FPG (mmol/L)	2 h-PG (mmol/L)	diabetes			CVD events		
		incidence (%)	RR (95%CI)	P value	incidence (%)	RR (95%CI)	P value
<5.6	<7.8	1.32%	1		4.93%	1	
<5.6	7.8–11.0	12.62%	9.52 (4.69–15.93)	<0.001	10.38% (1.32–3.36)	2.11	<0.01
5.6–6.0	<7.8	5.88%	4.44 (2.28–8.63)	<0.001	7.61%	1.54 (0.92–2.61)	NS
5.6–6.0	7.8–11.0	29.23%	22.06 (12.96–37.58)	<0.001	13.04% (1.40–5.01)	2.65	<0.01
6.1–6.9	<7.8	27.03%	20.40 (10.66–39.02)	<0.001	5.13% (0.47–7.25)	1.84	NS

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The impact of new diagnostic criteria for impaired fasting glucose

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Background and Aims: IFG is an abnormal metabolic state associated with an elevated risk of developing DM. The evidences from large studies showed that IFG defined as FBG 110–125 mg/dL failed to identify a number of individuals with IGT and increased risk for DM and cardiovascular disease. As a consequence, new diagnostic criteria for IFG, defined as FBG 100–125 mg/dL, were adopted. The aims of our study were to estimate the prevalence of IFG according to the new diagnostic criteria, to establish the concordance between the new proposed IFG category and IGT, to prospectively evaluate the progression to DM in persons with FBG 110–109 mg/dL vs. FBG 110–125 mg/dL and to determine the cardiovascular risk profile of these groups.
Materials and Methods: The study enrolled 1024 individuals, aged 40–70 years, without previous diagnosis DM, who underwent an OGTT. The subjects with IFG were followed over a period of 6 years. Plasma total cholesterol (TC), triglycerides (TG), HDLc, LDLc, systolic blood pressure (SBP), diastolic blood pressure (DBP) were compared in subjects with FBG 100–109 mg/dL vs. FBG 110–125 mg/dL.
Results: Lowering the threshold for IFG form 110 mg/dL to 100 mg/dL increased the prevalence of IFG from 8.01% to 22.85%. The new proposed IFG category identified 58.5% of all subjects with IGT compared to 26.3% with the old criteria. In subjects with FBG 110–125 mg/dL, the progression to DM was 34.15%, while in those with FBG 100–109 mg/dL it was 21.05%.

Table.1 Cardiovascular risk profile of subjects, according to FBG

Parameter	Subjects with FBG 100–109 mg/dL	Subjects with FBG 110–125 mg/dL	P
Number	152	82	–
Age(years)	55.45 ± 8.14	57.64 ± 7.52	NS
Sex M [n (%)]	75 (49.34)	40 (48.78)	NS
BMI (kg/m^2)	27.45 ± 5.23	29.23 ± 6.45	0.021
TC (mg/dL)	204.54 ± 21.31	214.32 ± 27.52	0.003
HDLc (mg/dL)	41.12 ± 6.21	39.54 ± 7.23	NS
LDLc (mg/dL)	122.31 ± 15.12	129.23 ± 16.32	0.001
TG (mg/dL)	215.32 ± 31.41	231.43 ± 32.24	<0.0001
SBP (mmHg)	133.45 ± 34.12	139.23 ± 29.23	NS
DBP (mmHg)	85.23 ± 16.14	89.56 ± 12.16	0.035

Data are means±SD. P was calculated with unpaired Student t test or with chi square test.

The age- and sex- adjusted incidence of major cardiovascular events (fatal and nonfatal myocardial infarction and cerebrovascular accidents) was 9.87/1000 person-years in subjects with FBG 100–109 mg/dL vs. 14.23/1000 person-years, in subjects with FBG 110–125 mg/dL, p=0.465 (0.56–3.73, 95% CI).

Conclusion: Lowering the threshold for IFG at 100 mg/dL will increase the prevalence of IFG approximately 3 fold. Although the cardiovascular risk profile was different in persons with FBG 100–109 mg/dL and FBG 110–125 mg/dL, the incidence of major cardiovascular events was similar in these groups. The new revised criteria for IFG allow the identification of a larger category of individuals with increased risk for DM and cardiovascular disease, than the old criteria, in which preventive strategies should be implemented.

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A world wide risk score for detecting individuals with impaired glucose metabolism or diabetes

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Background: Risk scores aiming at detecting people at high risk for diabetes are developed in white Caucasian Populations. These are not applicable in populations with diverse ethnic background due to different impact of the major risk factors on diabetes. Intervention studies in individuals with IGT decrease the development of diabetes. The diagnosis of IGT requires measurement of an OGTT, which is expensive and not feasible as screening. The objective is to develop a simple "world wide" risk score for detecting individuals with impaired glucose metabolism (IGM) or diabetes.
Methods: The risk score was derived using data from the DETECT-2 project, including 97,000 individuals in the age range 25–74 years covering all continents. All individuals without known diabetes underwent an OGTT. The DETECT-2 population is divided into geographical regions. Known and major risk factors for diabetes and IGM were analysed and ranked according to the area under the receiver operating characteristic curve (AUC).The highest ranked risk factors were then added one by one. Decision on whether a risk factor should be included in the final model was based on its contribution to the AUC. The independent variables were categorized using logistic regression analysis by region. For each variable in the multiple logistic regression analysis, a score was calculated by multiplying the regression coefficients by 10 and rounded to the nearest integer. A sum-score was calculated for each participant by summing the score up of the risk factors in the model.
Results: All regions, except for Europe Central and Africa ranked age and BMI as the two highest risk factors. The final model included Age, BMI and Gender. Adding on other risk factors did not increase the area under the curve by more than few percent.

Table: Performance characteristics for the final model by region.

Region	AUC	Cut-point value	Sensitivity	Specificity	PPV	% requiring further testing
Europe North	0.69	17	72.1	55.2	38.3	52.4
Europe Central	0.68	15	74.2	51.3	39.5	56.4
Europe South	0.67	9	78.7	43.9	35.8	62.5
North America	0.67	12	73.4	49.2	45.8	59.1
South America	0.66	11	70.1	54.3	32.4	51.5
Greenland	0.70	8	69.4	60.4	47.8	49.8
India	0.63	7	69.8	49.2	38.3	56.7
Japan	0.67	16	72.2	52.9	22.6	51.1
Asia remaining	0.69	14	71.0	56.0	33.2	50.4
Australia and New Zealand	0.73	18	75.0	59.5	33.6	47.9
Pacific Islands	0.66	13	80.2	40.1	44.4	67.5
East Med. and Middle East	0.69	18	70.5	58.8	60.2	55.0
Africa	0.67	11	71.4	54.2	31.9	51.8

Conclusion: A simple world wide risk score including three risk factors is developed. Using a risk score in a step wise screening for DM and IGM will reduce the numbers of FPG by 50% and detect 70% of all individuals with abnormal glucose tolerance.
Support:European Foundation for the Study of Diabetes(EFSD)-Pfizer Resource Award for Diabetes Research

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A diabetes risk score to identify high-risk individuals for type 2 diabetes in Asian Indians - the Chennai Urban Rural Epidemiology Study (CURES)

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Background and aim: A marked increase in the prevalence of diabetes has been documented in developing countries, particularly in India. Hence strategies to identify high-risk individuals would help to plan selective and cost effective screening. The aim of this study was to develop a Diabetes Risk Score [DRS] for identifying high-risk individuals.
Materials and Methods: The Chennai Urban Rural Epidemiology Study [CURES] is an ongoing epidemiological study in the Chennai city [formerly Madras], in South India. CURES recruited 26,001 individuals aged ≥20 years for Phase 1, based on systematic sampling technique so as to represent the population of Chennai. Every tenth subject recruited in Phase 1 of CURES was requested to participate in Phase 3 for screening of diabetes using World Health Organization [WHO] 2 hour venous plasma glucose criteria. The response rate was 90.4% (2350/2600). The Diabetes Risk Score was developed based on results of multiple logistic regression analysis, using diabetes as dependant variable and four risk factors [age, family history, physical inactivity and abdominal obesity] as independent variables. Based on the odds ratios obtained, a simplified score was created for each parameter: for an odds ratio (OR) between 1.1 to 1.9: the score given was 1, OR: 2.0 to 2.9, score of 2, OR 3.0 to 3.9, score of 3 and OR ≥ 4, score of 4. The Diabetes Risk Score was then determined by incorporating the scores for each factor into the model and adding the same for every study subject. Internal validation was performed on CURES data [Phase 1 and Phase 3] and external validation using data from an earlier study, the Chennai Urban Population Study [CUPS].
Results: Multiple logistic regression analysis revealed that age had the highest odds ratio for diabetes. The highest score was 8 and the least, 0. Internal validation of DRS revealed that the Area Under the Curve (AUC) for the ROC was 0.694 [95% Confidence interval [CI]: 0.659–0.730]. In the external validation, AUC for ROC was 0.758 [95% CI:0.696–0.820].

Sensitivity and specificity of different cutoff values of the DRS are given below:

Cut point	Sensitivity (%)	Specificity(%)	Positive predictive value (%)	Negative predictive value (%)
Diabetes diagnosed using WHO criteria in Phase 3 of CURES				
> 2	83.3	41.7	13.8	95.7
> 3	69.8	60.2	16.4	94.7
> 4	45.9	80.2	20.6	93.0
Diabetes diagnosed using WHO criteria [CUPS]				
> 2	85.5	39.7	7.4	98.4
> 3	82.7	61.0	10.4	98.5
> 4	59.6	79.1	13.5	97.3

Conclusion: A simple 4 point Diabetes Risk Score is a useful tool for identifying over 60% of newly detected Asian Indian diabetic subjects.

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Low physical fitness is associated with the metabolic syndrome – results from the PPP-Botnia Study (Prevalence, prediction and prevention of diabetes in the Botnia study)

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Background and Aims: A sedentary lifestyle has been connected with the rising prevalence of type 2 diabetes (DM2) and the cluster of cardiovascular risk factors named the metabolic syndrome (MSDR). Our aim was to assess the prevalence of diabetes, impaired glucose tolerance (IGT and IFG) and the metabolic syndrome in relation to physical fitness in the general population.
Materials and Methods: A population-based cohort of 5000 18–75-year-old subjects randomly selected from the Population Registry from Western Finland is invited to participate in the PPP-Botnia during 2004–2006. The study includes an oral glucose tolerance test (OGTT), serum lipid concentrations, BMI, waist circumference and blood pressure as well as a test for physical fitness (PFI) based upon walking time and change in heart rate during a 2 km walking test adjusted for age, sex and BMI. MSDR was defined according to the NCEP criteria.
Results: Among the first consecutive 1166 subjects studies 38 subjects had known DM2 and 27 new cases of DM2 were diagnosed. The prevalence of IGT in age groups 18–29 (n=214), 30–59 (n=517) and 60–75 yrs (n=435) was 1.4, 4.2, 14.5% and IFG in 3.3, 6.6 and 7.6%. The total prevalence of abnormal glucose tolerance (including DM) was 5.6%, 14.4% and 31.3% in the age-stratified groups. Thus the total prevalence of abnormal glucose tolerance was 12.5% and diabetes 5.6%. In the youngest age group the prevalence of MSDR was 9.2% in male and 4.8% in female subjects increasing to 18.2% and 21.2% in subjects aged 30–59 years and to 35.3 respectively 36.3% in the oldest age group. Physical fitness was assessed in 554 subjects. Subjects with very low PFI (n=107) had significantly higher frequency of both MSDR (34% vs. 5.2%, p<0.001) and all its components than those with normal or high PFI (n=230) (table).
Conclusion: An abnormal glucose tolerance was found in 12.5% and the diabetes prevalence was 5.6%. Low physical fitness was associated with glucose intolerance and the metabolic syndrome. The question that follows is how much should physical fitness be improved to reduce these risk factors.

	Very low PFI N=107	Normal or high PFI N=230	P-value
MSDR	34%	5.2%	<0.001
Abd. obesity	51.4%	6.1%	<0.001
Hypertension	72.0%	43.0%	<0.001
High Trigl. conc.	22.4%	9.1%	0.002
Low HDL conc.	40.2%	30.4%	0.08
IGT,IFG or DM2	17.8%	4.8%	<0.001

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High consumption of oral moist snuff („snus“) increases the risk of type 2 diabetes in a prospective study of middle-aged Swedish men

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Background: Cigarette smoking increases the risk of type 2 diabetes. Whether the alternative use of tobacco in the form of oral moist snuff, snus, also increases the risk of type 2 diabetes is disputed due to limited and somewhat conflicting data.
Subjects and Methods: The baseline investigation (1992–1994) comprised 3,128 healthy Swedish men, aged 35–56 yrs, living in the Stockholm area. They all underwent an oral glucose tolerance test (OGTT) and responded to a questionnaire regarding lifestyle. Of those not having diabetes at baseline (n=3,073), about 78% (n=2,392) were reinvestigated with a new OGTT and questionnaire 10 years later. The reinvestigation detected 4.2% (n=100) cases of previously undiagnosed type 2 diabetes. The odds ratio (OR) for type 2 diabetes was assessed only among those using snus at both initial and follow-up studies as compared to men who never had used snus. In addition, the OR for smoking was assessed. The consumption of boxes (à 50g) of snus/week or number of cigarettes/day was evaluated from the questionnaire at the follow-up study. The data were adjusted for major confounders, e. g. age, BMI, physical activity and family history of diabetes.
Results: The OR of developing type 2 diabetes during 10 years was not significantly increased in the group of snus users as a whole, OR 1.2 (95% confidence interval, CI, 0.7–2.1). However, the risk increased stepwise with increased weekly consumption of snus. Thus, ORs (CIs) for >=4 boxes/week of snus were 1.7 (0.8–3.4), >=5 boxes/week 2.3 (1.1-4.9), and >=6 boxes/week 3.6 (1.6–8.1). For comparison, men smoking at the initial study, and still smoking at follow-up had a significantly increased risk of developing diabetes compared to those never smoking, OR 2.0 (CI 1.1-4.0). This increased risk was most evident for those smoking >10 cigarettes daily, OR 2.4 (CI 1.1-5.0).
Conclusion: This ten-year prospective study of middle-aged men shows that both high-consumers of snus (oral moist snuff), and smokers, are at increased risk of developing type 2 diabetes. Both smoking and snuffing entails chronic exposure to nicotine, hence the diabetogenic effects of tobacco use is likely caused by direct and/or indirect effects of nicotine. Thus, augmented catecholamine levels due to tobacco use could contribute to development of diabetes.
Support: Stockholm County Council, Swedish Council for Working Life and Social Research, Glaxo Smithkline, Sweden

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Non-genetic determinants of type 2 diabetes

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Effect of alpha-tocopherol and beta-carotene supplementation on the incidence of type 2 diabetes in Finnish male smokers

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Background and Aims: Type 2 diabetes is associated with reduced antioxidant defenses. Only a few studies in humans have emerged about the preventive effect of antioxidants on diabetes. The aim of this study was to determine whether alpha-tocopherol and beta-carotene supplementation would reduce the incidence of type 2 diabetes in Finnish male smokers.
Materials and Methods: The Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study, a randomized, double-blind, placebo-controlled trial with 2 x 2 factorial design, randomized 29 133 male smokers aged 50–69 years to receive either vitamin E 50 mg/d, or beta-carotene 20 mg/d, or both, or placebo for a median of 6.1 years. During the follow-up (intervention plus 4.5 years postintervention) 1187 incident cases of diabetes were observed among the 27 861 men not reporting diabetes at baseline. The main outcome was drug-treated diabetes identified from a nationwide registry of patients receiving drug reimbursement.
Results: The relative risk for diabetes between alpha-tocopherol recipients and non-recipients was reduced (RR 0.87 95%CI,0.77–0.98) when excluding first two follow-up years. In participants with no clinical abnormalities for metabolic syndrome, the preventive effect of supplemental alpha-tocopherol was stronger (RR 0.64 95%CI,0.41–0.98). Supplemental beta-carotene had no effect on diabetes.
Conclusion: Since type 2 diabetes is an increasing health problem with many severe complications even a small preventive effect of alpha-tocopherol could have notable public health impact. However, more conclusive studies are needed before specific measures to increase the intake of vitamin E are justified. In the meantime it is prudent to consume a lot of vegetables and fruits, foods associated with a healthy life.
The ATBC Study was supported by a contract N01-RC-37004 with National Cancer Institute, Bethesda, MD, USA

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Association of pulse pressure with fasting hyperglycemia in the general population

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Background and Aims: Hypertension is an established key component of metabolic syndrome (MS) associated to obesity, in particular visceral obesity. Pulse pressure (PP), a measure of arterial stiffness, is associated to obesity, hypertension, and diabetes. Aims of this study is to evaluate the prevalence of PP in the diagnosis of MS, its association with the MS impairments, and the categories of glucose tolerance.
Materials and Methods: A cohort of inhabitants of Florence, Italy, participating to a screening study for diabetes. 3112 (1748 F, 1364 M) subjects, with no history of diabetes, aged (mean±SD) 55.2 ± 11.5 yrs, with a BMI 26.0 ± 4.2 kg/m^2, waist circumference 84.3 ± 11.4 cm in F, and 97.6 ± 10.3 cm in M were studied. After overnight fasting (>8 hrs) blood samples for glycemia, total and HDL cholesterol, triglyceride were collected; those subjects with a fasting plasma glucose (FPG)<126 mg/dl underwent a standard OGTT (75 g); anthropometric and clinical parameters as systolic, diastolic, PP, and mean blood pressure (MBP) were measured. MS was defined according to National Cholesterol Education Program (NCEP) criteria.
Results: 2134 (75%) subjects presented normal glucose tolerance, 272 (8.7%) impaired glucose tolerance (IGT), 186 (6.0%) impaired fasting glucose (IFG), and 209 (6.7%) were affected by diabetes mellitus (DM). According to NCEP criteria 541 (17.4%) individuals showed fasting plasma glucose ≥110 mg/dl, 1178 (37.8%) hypertension, 957 (30.7%) pathological waist circumference (waist>88 cm in F and>102 cm in M), 416 (13.4%) low HDL cholesterol, and 631 (20.3%) hypertriglyceridemia; 492 (15.8%) of subjects resulted affected by MS. PP values resulted 45.8 ± 13.0 mmHg, and MBP values 109.1 ± 14.0 mmHg. Subdividing PP and MBP in quintiles, it was observed a progressive increase of prevalence of each MS impairment from the 1st to 5th quintile. In particular, prevalence of diabetes was 7 fold increased from the 1st to 5th quintile of PP versus 2 fold and 3 fold increase of prevalence of IGT, and IFG, respectively. At multivariate analysis, PP was significantly ($p<0.01$) associated with FPG even after adjustment for sex, age, BMI, and mean blood pressure.
Conclusion: Elevated pulse pressure is associated with components of the metabolic syndrome, and with fasting hyperglycaemia in particular, independent of high mean blood pressure, age, and adiposity.

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The correlation between reactions to the stress and the age of onset of type 2 diabetes

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Background and Aims: Stress is considered as one of the factors related to the development of Type 2 diabetes. However, severity of reactions to the stress may be various between individuals. The aim of the study was to clarify whether the severity of reactions to the stress affects to development (earlier disease manifestation) of Type 2 diabetes.
Materials and Methods: Sixty seven Type 2 diabetic patients (18 males; mean age 61,4 ± 7,4 years; mean diabetes duration 9,8 ± 13,4 years; mean HbA1c level 8,3 ± 1,9%) reported about severity of the reaction to 10 typical stressful life events (SLE) during their adult life before the onset of diabetes: death of a husband/wife, divorce, separation from husband/wife, imprisonment, death of a relative, serious illness, marriage, dismissal, reconciliation with a husband/wife, retirement. To evaluate the stress severity visual analog scale (VAS; 0–10 score) was applied. The patients were divided into two groups according the ratio: summarized VAS score/total number of SLE. Group 1 consists of patients with high ratio (> mean value) or with "severe" reaction to the stress. Group 2 consists of ones with low ratio (< = mean value) or with "mild" reaction to the stress. The age of the diabetes onset was compared.
Results: Mean number of SLE for all patients was 4,3 ± 1,7; mean summarized VAS score - 36,2 ± 17,7; mean VAS/SLE ratio value 8,4 ± 4,1. Mean age of the onset of Type 2 diabetes in patients of group 1 ("severe" reaction to the stress, n=32) was younger than in patients of group 2 ("mild" reaction to the stress, n=35): 49,5 ± 9,5 vs 54,2 ± 9,0 (Mann-Whitney U test p=0,05). Besides that the number of patients with the onset of coronary heart disease (CHD) before 55 years old in group 1 was significantly higher (5 vs 0; p<0,02).
Conclusion: These data demonstrate that "severe" reaction to the SLE is associated with earlier onset of Type 2 diabetes. The individuals who react to stress more intensive probably represent the group of high risk of early Type 2 diabetes manifestation. The problem needs follow investigation with relation to both Type 2 diabetes and cardiovascular diseases.

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Occupational stress and low emotional support increase synergistically the risk for future type 2 diabetes in middle-aged women

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Background and aims: Psychosocial stress and depressive symptoms are shown to be risk factors for development of type 2 diabetes. The biological mechanisms explaining this diabetogenic effect include behavioural aspects such as obesity, physical inactivity, smoking and unhealthy drinking and also neuroendocrine pathways, with activation of the hypothalamus-pituitary-adrenal (HPA) axis and increased levels of stress hormones, leading to visceral obesity and insulin resistance. However, the origin of the psychosocial stressors, that are players on these biological systems, are not as well described. Our aim was to investigate the association between psychosocial conditions and life style and future development of type 2 diabetes in middle-aged men and women.
Material and methods: A prospective case-referent study nested within an ongoing population based health survey in primary care in northern Sweden. All participants in the health survey (n=33,336) in the city of Umeå with surrounding municipalities (135 000 inhabitants) were included. 237 cases, initially non-diabetic but diagnosed with type 2 diabetes after 5.4 ± 2.6 years, were identified and two age- and sex-matched referents, non-diabetic after 8.1 ± 2.5 years, for each case. Occupational stress,

analysed by the demand/control model (DCM), social integration and emotional support, education, civil status, smoking and physical activity as well as BMI and heredity for type 2 diabetes were evaluated with respect to subsequent clinical diagnosis of type 2 diabetes by regression analysis.
Results: A passive or a tense work situation and low emotional support were associated with future diabetes with OR 1.8 (CI95% 0.65–4.89), 3.3 (1.14–9.30) and 2.0 (0.97–4.17) respectively and interaction variables with low emotional support combined with a passive or a tense work situation displayed OR 4.2(1.06–16.81) and 9.5 (1.83–49.32) respectively in women. In men high emotional support combined with a passive work situation displayed OR 0.3 (0.11–0.97). BMI and heredity for type 2 diabetes were strongly predictive in both genders and in men in addition smoking.
Conclusion: There is an independent association between occupational stress and future development of type 2 diabetes in women. Low emotional support is a strong modifier that increases this risk synergistically in the development of T2DM. These findings are important for clinicians to take into account when evaluating the risk for future development of T2DM in their patients.

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Obesity in 15-year-old adolescents is related to oxidative stress and inflammation
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Background and Aims: Obesity is strongly associated with the metabolic syndrome, which, in turn, is associated with low-grade inflammation. Inflammatory factors (e. g., cytokines and adipokines) originating from obesity-induced visceral fat may produce oxidative stress that has been associated with insulin resistance and cardiovascular risk. Very little is known about the relations among these factors in adolescents.
Materials and Methods: Urinary levels of 8-iso-$PGF_{2\alpha}$, a major F_2-isoprostane and 15-keto-dihydro-$PGF_{2\alpha}$, a major metabolite of $PGF_{2\alpha}$, were measured in 274 13–17 year old adolescents (M=153; F=121) as indicators of oxidative stress and cyclooxygenase-mediated inflammation, respectively. The subjects were participating in a study of the metabolic syndrome and underwent anthropometric measurements and a euglycemic insulin clamp at which time blood was obtained for fasting insulin, glucose and lipids and an overnight urinary sample was collected.
Results: F_2-isoprostane levels were significantly correlated with BMI (r=0.13; p=0.03), waist circumference (0.16; 0.009), fasting insulin (0.13; 0.03) and glucose (0.12; 0.04). $PGF_{2\alpha}$ metabolite were significantly correlated with BMI (0.14; 0.02), waist circumference (0.15; 0.01) and fasting insulin (0.12; 0.04). Among the boys, F_2-isoprostane was correlated with waist and glucose and$PGF_{2\alpha}$ metabolite was correlated with waist and insulin. These relationships were not significant after adjustment for BMI. Children in the highest quartile of BMI and waist circumference had the highest levels of F_2-isoprostane and $PGF_{2\alpha}$ metabolite levels.
Conclusion: These data show that a significant relation between obesity and both oxidative stress and prostaglandin mediated inflammation are already present in adolescence thus further suggesting the importance of preventing obesity early in life.
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Low birthweight and markers of inflammation and endothelial activation in adults - the ARIC study
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Background and Aims: To test the hypothesis that low birthweight, reflecting unfavourable stimuli during a critical prenatal period, might produce a longstanding proinflammatory tendency, we investigated whether low birthweight is associated with alteration in blood levels of markers of inflammation and endothelial activation in middle-aged adults.
Materials and Methods: The Atherosclerosis Risk in Communities (ARIC) Study enrolled white and African-American subjects aged 45–64 years sampled from four U.S. communities. Participants were asked their birthweight. Respondents indicating qualitatively a low birthweight or quantitatively a birthweight <2500g were classified as having low birthweight. An inflammation/endothelial activation score from 0 to 6 was created, one point being given for each above-median value of white blood cell count, fibrinogen, von Willebrand factor and Factor VIII, and for each below-median value of albumin and aPTT. Multiple logistic regression evaluated the adjusted association of low birthweight with a high (≥ 4 points) score.
Results: Of the 10555 individuals reporting birthweight and with available information on all markers and covariates, 361 (3.6%) reported low birthweight. The mean (standard deviation) score was 3.5 (1.4) for those with low birthweight and 3.1 (1.6) for those in other birthweight groups (P<0.001). In logistic regression models adjusted for gender, ethnicity, age, study center, educational level, and current drinking and smoking status, those with low birthweight had an increased odds of having a high score (OR = 1.35, 95% CI = 1.09–1.68) compared to participants with normal or high birthweight. Additional adjustment for BMI and WHR modestly increased the odds ratio for the overall association (OR=1.43, 95% CI = 1.15–1.79).
Conclusion: In the ARIC Study, low birthweight predicted greater inflammation and endothelial activation, as indicated by the higher score of blood markers, consistent with the hypothesis that fetal stressors may result in a hyper-responsive innate immune system. This could be mediated by altered gene expression (fetal programming). Such a pro-inflammatory tendency could help explain the association of low birthweight with elements of the metabolic syndrome and atherosclerosis.
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Multivariate genetic analyses of phenotypes related to the metabolic syndrome

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Background and Aims: Insulin resistance, impaired glucose tolerance, obesity, hypertension and dyslipidemia are phenotypes described as part of the metabolic syndrome. We investigated genetic and environmental contributions to the syndrome related traits by analysing information from Danish healthy twins. The aim was to investigate whether there is a genetic correlation between phenotypes, i. e. whether the same gene or genes can underlie these phenotypes.
Materials and Methods: 756 twin pairs (311 monozygotic and 435 dizygotic) from the Danish Twin Registry took part in a clinical investigation comprising an oral glucose tolerance test, measurements of anthropometry, lipids and blood pressure. Univariate and multivariate models were fitted with residual maximum likelihood using mixed linear models. To minimize standard errors of estimated genetic correlations all data were analyzed simultaneously fitting sex and age as covariates.
Results: From the univariate analyses heritability estimates of BMI, waist, HDL, systolic and diastolic blood pressure were larger than 0.50. Triglycerides, 120 minutes glucose and fasting insulin were between 0.30 and 0.50. Estimates of a common environmental component were small. Multivariate analyses revealed genetic correlations around 0.5 between BMI, waist and fasting insulin and waist and fasting insulin. Genetic correlations between either waist, BMI or fasting insulin and either systolic or diastolic blood pressure or triglycerides were around 0.25. For the remaining combinations of phenotypes associated with the metabolic syndrome estimated genetic correlation under 0.20.
Conclusion: The results of this analysis demonstrate that it is quite possible that several of the phenotypes associated with the metabolic syndrome shares one or more genes of etiological importance.

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Is there a common genetic background for the metabolic syndrome using different definitions?

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Background and Aims: The metabolic syndrome (MSDR) is a multifactorial cluster of disease-related components, which to 30–60% are attributable to genetic factors. At least two definitions are commonly used for MSDR: WHO98 and NCEP. However, the genetic causes of MSDR are largely unknown, as well as whether the different definitions capture clusters of similar genetic background. The aim of this study was therefore to study association between variants in a number of putative candidate genes and MSDR defined either by WHO98 or NCEP.
Materials and Methods: 4663 individuals from the Botnia study were included. Using the WHO98 definition, 1765 had MSDR (m/f 894/870, age 64 ± 13 y, 61.8% T2D, BMI 29.0 ± 4.5 kg/m^2) while 2577 did not fulfill criteria and served as controls (CONTR) (m/f 1108/1466, age 55 ± 13 y, BMI 25.4 ± 3.5 kg/m^2). The corresponding figures using the NCEP definition were: 1728 MSDR (m/f 744/984, age 64 ± 13 y, 62.8% T2D, BMI 29.4 ± 4.5 kg/m^2) and 2617 CONTR (m/f 1253/1360, age 54 ± 13 y, BMI 25.2 ± 3.5 kg/m^2) individuals. The variants in *UCP2* (-866 C/T), *IRS1* (Gly972Arg), *PPARG* (Pro12Ala) and *PGC1α* (Gly482Ser) genes were genotyped by allelic discrimination on ABI 7900. The variants in β*1AR* (Gly389Arg), β*2AR* (Arg16Gly and Gln27Glu), β*3AR* (Trp64Arg), *CAPN10* (SNP43 G/A and 44 C/T), *GYS1* (XbaI C/T) and *APM1* (SNP-4041 A/C, SNP276 G/T, SNP2019 Ins/Del) genes were genotyped by single base-pair extension on ABIprism 3100.
Results: Using the WHO98 definition, significant differences in genotype frequency distribution between MSDR and CONTR were found for two SNPs in the β*2AR* gene: Arg16Gly (15.2%, 48.3%, 36.6% vs. 17.7%, 44.1%, 38.2% for Arg/Arg, Arg/Gly, Gly/Gly, p=0.019) and Gln27Glu (35.2%, 50.3%, 14.4% vs. 39.2%, 44.6%, 16.2% for Gln/Gln, Gln/Glu, Glu/Glu, p=0.0027).
For NCEP significant differences in genotype frequencies between MSDR and CONTR were seen β*2AR* Gln27Glu (36.2%, 49.6%, 14.2% vs. 38.8%, 44.9%, 16.3% for Gln/Gln, Gln/Glu, Glu/Glu, p=0.013) and *APM1* SNP-4041 A/C (48.0%, 39.3%, 12.7% vs. 45.1%, 43.9%, 11.0% for A/A, A/C, C/C, p=0.016).
Conclusion: Variants in the β*2AR* gene are associated with MSDR independently of the definition used and thus β*2AR* can be considered as strong candidate gene for the syndrome.

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Coincident linkage of different metabolic syndrome related phenotypes and chromosome $2q_{12}$

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Background and Aims: to test if a region on chromosome $2q_{12}$ previously linked to hypertension and other metabolic syndrome phenotypes in genome wide scans, is linked to quantitative phenotypes related to the metabolic syndrome in Swedish healthy siblings.
Materials and Methods: We have phenotyped 260 healthy, normotensive siblings belonging to 118 nuclear families from Malmö by measuring 24-hours Ambulatory Blood Pressure Monitoring, anthropometric and metabolic parameters related to the metabolic syndrome. Families were ascertained from the population based „Malmö Preventive Project“ and „Malmö Diet and Cancer“ studies. We conducted a fine mapping of the region on chromosome 2 spanning between 107 to 130 cM using 11 informative polymorphic markers (average maximal heterozigosity 81%; mean distance between the markers 2,5 cM). Heritability (H_2R) was estimated and variance-component linkage analysis was performed for each blood pressure parameter, metabolic syndrome related phenotype and a sum of standardized metabolic syndrome phenotypes (the SSMSP formed by 24-hour SBP + glucose - HDL-cholesterol + triglycerides + Waist/Hip ratio) as quantitative traits after adjustment for significant covariates using „Solar” software package.
Results: All the metabolic syndrome variables and the SSMSP resulted to be heritable traits in our family collection (table 1). For 24-hours and day-time and pulse pressure (PP) LOD score >1 has been found all over the region between 107 to 123 cM (between markers D2S2232 and D2S2269) with a maximum peak respectively of 2,6 at 115 cM and of 1,6 at 119 cM. For the waist-hip ratio LOD score >1 has been found between 107 to 126 cM (maximum peak 2,3 at 107 cM) and for the SSMSP between 113 and 119 cM (maximum peak 1,5 at 117 cM) (table 1). No LOD score >1 has been found for systolic blood pressure (SBP), diastolic blood pressure (DBP) and the other metabolic syndrome phenotypes analyzed.
Conclusion: Not only the metabolic syndrome parameters separately but also a compound sum of metabolic syndrome related phenotypes (SSMSP) are heritable traits suggesting a genetic component subtending the entire metabolic syndrome. The linkage analysis results suggest that chromosome $2q_{12}$ could harbour one or more genes implied in blood pressure homeostasis and metabolic syndrome development. (Alpha)2B adrenoceptor gene that maps in this region, at about 115 cM, is a potential candidate gene.

Heritability and linkage analysis on chromosome 2q12 of the metabolic syndrome phenotypes

Phenotypes	Adjustment	H2R	p-value	Maximum LOD	Genetic distance (cM)
Waist/hip ratio	Age, sex	0,60	<0,001	2,3	107
Body Mass Index	Age, sex	0,57	<0,001	n.s.	n.s.
Total Cholesterol	Age, sex	0,62	<0,001	n.s.	n.s.
HDL-Cholesterol	Age, sex	0,59	<0,001	n.s.	n.s.
Triglycerides	Age, sex	0,52	<0,001	n.s.	n.s.
Glucose	Age, sex	0,44	<0,01	n.s.	n.s.
Insulin	Age, sex	0,30	<0,05	n.s.	n.s.
24-hour SBP	Age, sex, BMI, 24-hour Heart Rate	0,43	<0,05	n.s.	n.s.
24-hour DBP	Age, sex, BMI, 24-hour Heart Rate	0,48	<0,01	n.s.	n.s.
24-hour PP	Age, sex, BMI, 24-hour Heart Rate	0,70	<0,001	2,6	115
Compound SSMSP	Age, sex	0,64	<0,001	1,5	117

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Relationship between common variants in the *Stearoyl CoA desaturase (SCD)* gene and intermediate phenotypes associated with type 2 diabetes

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Background and Aims: Stearoyl CoA desaturase (SCD) is the key final step in de novo lipogenesis, and the subject of increasing interest with regards to the pathogenesis of type 2 diabetes. *Scd*-null mice displays increased insulin sensitivity and resistance to obesity and diabetes. The consequences of variation in SCD activity in man are however controversial.
The purpose of this study was to study the relationship between sequence variation in *SCD* and diabetes-related metabolic traits.
Materials and Methods: For the study, 780 individuals from Oxford Biobank were selected. This is a random, population-based collection of healthy men and women in the age range of 30–50 years, who have previously undergone a detailed baseline screening process with measurements of fasting serum insulin, fasting glucose and metabolic and anthropometric variables related to the insulin resistance syndrome. Eight SNPs within the *SCD* gene were selected for genotyping: five are haplotype tagging SNPs derived from the HAPMAP project, the other three SNPs were selected on the basis of our own previous resequencing data. One of these is a non-synonymous SNP in exon 5 (M224L). Genotype-phenotype correlations were investigated through single point and multipoint analysis of quantitative intermediate traits.
Results: Carriers of the L allele (frequency of L = 0.43) at M224L (rs11598233) had a significantly higher mean BMI than MM homozygotes (geometric mean [SD range]: 25.8 [22.0–30.4] kgm^{-2} vs. 25.1 [21.7–29.2] kgm^{-2}, p=0.027). They also had a lower HDL cholesterol (1.32 [1.05–1.66] mmol/l vs 1.41 [1.11–1.79] mmol/l, p=0.0008). This effect appeared to be particularly strong among women. Similar findings were observed at a second SNP in the promoter (rs2275656 at position -964) where a significantly higher mean BMI (25.7 [21.9–30.1] kgm^{-2} vs. 24.8 [21.6–28.6] kgm^{-2}, p=0.048) and a lower HDL cholesterol (1.34, [1.06–1.69] mmol/l vs. 1.42 [1.12–1.79] mmol/l, p=0.021) were observed in the carriers of a G allele (frequency of G =0.62) compared to CC homozygotes. Haplotypic analyses (using haplotype trend regression) confirmed these findings but did not reveal any additional haplotypic associations.
Conclusion: The study suggests that variation in the *SCD* gene is related to diabetes-related intermediate traits. Lower HDL cholesterol and a higher BMI are characteristic features of the metabolic syndrome associated with higher risk of type 2 diabetes, and this study suggests that a proportion of the individual susceptibility to type 2 diabetes may be conveyed by variation in the *SCD* gene.
Support: Diabetes UK Scholarship

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Effect of the Val1483Ile polymorphism in the fatty acid synthase gene (*FAS*) on obesity in children and adolescents from Germany

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Background and Aims: Inhibition of fatty acid synthase (FAS) induces a rapid decline in fat stores in mice, suggesting its role in energy homeostasis. The human fatty acid synthase gene (*FAS*) maps to chromosome 17q25, a region showing suggestive linkage with adiposity in a genome wide scan for genetic determinants of type 2 diabetes mellitus (T2DM) and obesity in Pima Indians. Recent studies in Pima Indians identified a Val11483Ile polymorphism, that was significantly associated with percentage of body fat and substrate oxidation rates.
Materials and Methods: We investigated the effect of Val1483Ile polymorphism on obesity (characterized by body mass index - BMI) in 737 Caucasian children and adolescents from Germany (380 girls and 357 boys). This cohort is part of the Leipzig Schoolchildren Project, which involves 2500 children from the city of Leipzig, Germany, at age 6 to 17 years recruited in 1999–2001. The Val1483Ile polymorphism was genotyped in all 737 subjects using the TaqMan allelic discrimination assay (Applied Biosystems, Inc).
Results: Frequency of the Ile-allele was 0.03, which was substantially lower than the one observed previously in Pima Indians (0.10). The genotype distribution was in Hardy-Weinberg equilibrium (P=0.38). The effect of the variant on BMI was evaluated using general linear regression models. No association of the Val1483Ile with BMI was found (P=0.95). However, based on interaction between gender and genotype (P=0.004), the analysis was also carried out in males and females separately. Boys with Ile/x had a lower mean BMI-SDS than Val homozygotes (–0.358 ± 0.292 vs. 0.094 ± 0.053, P=0.04), while an opposite effect was observed in girls (0.484 ± 0.180 vs. 0.087 ± 0.051, P=0.04).
Conclusion: Consistent with findings in Pima Indians, our results suggest that the Val1483Ile mutation of *FAS* protects male Caucasian children against the development of obesity.

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A non-synonymous substitution (R1467H) in ARHGEF11 is associated with insulin resistance and type 2 diabetes in Pima Indians

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A prior genome-wide linkage scan in Pima Indians indicated a young onset (age<45 years) type 2 diabetes mellitus (T2DM) susceptibility locus on chromosome 1q21-23. The region of linkage spans 24Mb. *ARHGEF11,* which encodes the Rho guanine nucleotide exchange factor 11, was analyzed as a positional candidate gene for this linkage because this protein may form a complex with G proteins and stimulate Rho-dependent signals, such as the insulin signaling cascade. Sequencing of the 41 exons, exon-intron boundary regions, and 2 kb of the 5' (putative promoter) region of *ARHGEF11* in 24 non-first degree related Pima Indians identified 27 variants, three of which were non-synonymous amino acid substitutions (R293Q, S1456G, and R1467H). These variants were genotyped, for association analysis, in the same Pima Indian subjects that were used for the T2DM linkage study (N=1300). The R1467H was associated with young onset diabetes (P=0.01, odds ratio=3.3, CI=1.31-8.59, additive model) after adjusting for sex and Pima heritage. The risk allele H had a frequency of 0.10. In a subgroup of 355 non-diabetic Pimas who have undergone detailed metabolic testing including measurements of body composition, OGTT, insulin secretion measurements, and a hyperinsulinemic-euglycemic clamp, the risk allele H was associated with higher insulin concentrations (log transformed) at 120 min following a 75 g OGTT (2.18/2.20/2.92 for RR/RH/HH respectively, P<0.05, additive model;) and lower glucose disposal rates (log transformed) during the physiologic dose of insulin during a hyperinsulinemic-euglycemic clamp (0.55/0.54/0.43 for RR/RH/HH respectively, P=0.02, additive model) after adjusting for age, sex, percentage of body fat, and Pima heritage. These studies suggest that the H allele at R1467H is associated with increased risk of type 2 diabetes as a result of increased insulin resistance. Replications of these findings are currently being attempted in other populations.

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The association of transcription factor 1 (TCF1) polymorphisms with features of the insulin resistance syndrome

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Background and Aims: A large proportion of Maturity Onset Diabetes of the Young can be accounted for by mutations in the *TCF1* gene. It has also been hypothesised that *TCF1* polymorphisms may contribute to the pathogenesis of type 2 diabetes mellitus and its complications. The aims of this study were to screen 3 Kb of the *TCF1* 5' flanking region for novel polymorphisms, to determine their influence on promoter function and to evaluate their relationship to cardiovascular and insulin resistance related variables.
Materials and Methods: Laboratory Studies: We screened the *TCF1* 5' flanking region (–2800 to + 96 bp) in 95 blood donor DNA samples by denaturing HPLC and characterised the polymorphisms by sequencing. The influence of the novel polymorphisms on promoter activity was assessed with luciferase reporter gene constructs containing the 4 most common haplotypes of the *TCF1* 5' flanking region, which were transiently transfected into HepG2 cells. Luciferase activity was determined using the Dual Luciferase Reporter Assay System (Promega). Clinical Studies: Stored DNA from 508 subjects from the Leeds Family Study were genotyped for the identified polymorphisms and the previously identified I27L polymorphism. Statistical analyses: The relationships of genotype to phenotype was assessed by SPSS (SPSS Inc) and SOLAR (Southwest Foundation for Biomedical Research) software packages. SOLAR analysis provided an assessment of the contribution of polymorphisms to variance in phenotypes.
Results: Laboratory studies: Eight polymorphisms were identified in the *TCF1* promoter, 7 of which were novel (TGGGGGT-del at -160; -257 A/G; -1182 T/C; -1279 A/G; CT-del at -1447; -1657 G/A; -1697 C/T and -2137 C/A). Luciferase reporter gene analysis of the four common haplotypes indicated that the TAGA haplotype (-1182 T; -1279 A; -1657 G; -2137 A) exhibited a significant increase in promoter activity of 30% ($p < 0.05$) when compared to the other three common haplotypes (TAGC, CGAC, and CGGC). Clinical Studies: SOLAR analysis indicated that the -1182 T/C and -1279 A/G polymorphisms accounted for 2.1% (p= 0.033) of the variance in fasting glucose. The -1657 G/A polymorphism accounted for 1.1% (p= 0.032) of the variance in fasting cholesterol. The previously identified I27L mutation explained 0.8% (p=0.066) of the variance in LDL and 1% (p= 0.032) of the variance in total cholesterol.
Conclusion: This study has shown that polymorphisms in the *TCF1* promoter have a small but significant influence on fasting cholesterol and glucose levels.
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The promoter region of adiponectin gene is a determinant in modulating insulin sensitivity in childhood obesity

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Background and Aims: Multiple mechanisms are thought to contribute to the pathogenesis of insulin resistance. Among these the role of adipose tissue, obesity and genetic factors seems to be of great significance. Genes playing a role in adipose tissue metabolism can be considered as possible responsible of insulin sensitivity. Previous data suggest that polymorphisms in the adiponectin, encoding a novel adipose-derived hormone, were associated with insulin resistance. We investigated the role of -11377G>C, -11391A>G, +45T>G, +276G>T SNPs and haplotypes of adiponectin gene with metabolic abnormalities of insulin resistance in a population of children (n=220, mean age 10.38 ±SD 2.8).
Materials and Methods: The -11391 G>A, +45 T>G and +276 G>T SNPs were genotyped using the flurogenic 5' nuclease assay application of the ABI PRISM 7900 HT Sequence Detection System and the -11377 C>G SNP was typed by PCR followed by SnapShot ddNTP Primer Extension Kit (Applied Biosystems).
The effect of the polymorphisms on quantitative variables was investigated using multiple linear regression analysis. Values were adjusted for BMI-SDS and pubertal stage; each polymorphism was introduced as a dichotomous variable in the analysis. To perform Haplotype analysis we used THESIAS program.
Results: Children carrying -11391G and -11377G alleles have higher fasting insulin levels and HOMA-IR index compared to non carriers (p=0.0003, p=0.002 and p=0.0006, $p<0.0001$, respectively). Moreover -11377G carriers showed higher triglycerides and fasting glucose levels compared to non carriers (p=0.00064 and $p<0.0001$). The +45G variant shows higher fasting glucose and 2 h glucose levels when compared to TT genotype (p=0.0045 and p=0.0008). We observed that -11391^{G}-11377^{G} 45^{T}+276^{G} haplotype shows higher fasting insulin levels and HOMA-IR index (p=0.001 for both) compared to all the others investigated haplotypes.
Conclusion: Our findings suggest that although -11391G>A, -11377C>G and +45T>G SNPs are in positive linkage disequilibrium, they independently influence the studied phenotypes, and that the effect of + 45T>G SNP is at best marginal compared with the promoter SNPs.

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Insulin resistance candidate genes and cholesterol levels in juvenile obesity: role of *PPARG* Pro12Ala and *APM1* G276T

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Background and Aims: Obesity and type 2 diabetes are prevalent risk factors for cardiovascular disease (CAD). Variation in several genes has been associated with both these conditions along with insulin resistance, low high-density lipoprotein (HDL) cholesterol and high triglyceride levels. Less is known about the possible role of these genes for total and low-density lipoprotein (LDL) cholesterol elevation, two of the strongest risk factors for CAD.
Materials and Methods: 285 morbidly obese children and adolescents and 2.470 non-diabetic adult subjects were genotyped for single nucleotide polymorphisms in the genes encoding the transcription factor peroxisome proliferator-activated receptor-γ2 (*PPARG* Pro12Ala), the adipocyte hormone adiponectin (*APM1* G276T), and the cholesterol determinant apolipoprotein E (*ApoE*-epsilon). Phenotypic differences between juvenile genotype carriers were investigated, focusing on the effect on lipid levels, controlling for the known influence of *ApoE*. A risk genotype combination was identified by analysis of covariance. The effect of individual genotypes, the risk genotype combination, and the protective inverse of this combination, were then investigated in the adult population.
Results: Juvenile homozygous wild type carriers of *PPARG* Pro12Ala had significantly higher total (p=0.03) and LDL-cholesterol levels (p=0.02) than carriers of the variant Ala-allele (adjusted for age, gender, body mass index, insulin sensitivity, and ApoE phenotype). Homozygous carriers of the variant allele of *APM1* G276T had significantly higher total (p=0.00009) and LDL-cholesterol levels (p=0.0009) than carriers of the wild type allele. The ApoE phenotype (2/2 or 2/3 vs. 3/3 vs. 3/4 or 4/4) also had a significant effect on both total (p=0.0004) and LDL-cholesterol levels (p=0.00001). Juvenile subjects carrying the combination *PPARG* Pro/Pro and *APM1* T/T had significantly higher total (p=0.00002) and LDL-cholesterol levels (p=0.0001) than carriers of any other combination, independently of the respective participating risk genotypes. Conversely, carriers of the inverse genotype combination (Pro/Ala or Ala/Ala and G/G or G/T) had significantly lower total (p=0.036) and LDL-cholesterol levels (p=0.017), although this effect did not persist after adjusting for *PPARG* genotype. The association between *PPARG* Pro/Pro and total cholesterol was confirmed in the adult population ($p<0.05$), as was the expected difference between *PPARG* Pro12Ala wild type and variant allele carriers concerning fasting triglycerides (p=0.0025). The presence of a protective genotype combination was also confirmed ($p<0.05$ and 0.05 for total and LDL-cholesterol, respectively) but again, outside of the ApoE reference group (ApoE 3/3) this effect did not persist after adjusting for *PPARG* genotype.
Conclusion: Genetic variants in candidate genes for insulin resistance are associated with cholesterol levels and offer additional information to the assessment of subjects at risk of developing cardiovascular disease.

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Genetics of insulin resistance II

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Isolation of a genetic region conferring skeletal muscle-specific insulin resistance in a spontaneously hypertensive rat-derived congenic strain
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Background and Aims: The spontaneously hypertensive rat (SHR) and the polydactylous rat (PD/Cub) are models of human metabolic syndrome (MS). In several human and rat linkage and association studies, features of MS were found to be connected with human chromosome (chr.) 11q23, syntenic to rat chr. 8q22. This segment, when introgressed from PD/Cub (including the *Plzf* gene most likely responsible for the *Lx* mutation in PD/Cub) onto the genetic background of SHR, was previously shown to influence most of the MS attributes in SHR-*Lx* congenic strain. In the process of narrowing down the segment, the SHR-*Lx PD5* congenic substrain [SHR.PD(*D8Rat43-D8Rat94*)/Cub, *RGD ID: 1302816*] was derived.
Materials and Methods: In order to determine the extent of the differential segment of PD/Cub origin in SHR-*Lx* PD5 strain we employed series of microsatellite markers densely covering the genomic region in question. Male SHR (n=13) and SHR-*Lx* PD5 (n=10) rats were fed standard laboratory chow *ad libitum*. At the age of 5 months, dexamethasone (DEX) was administered in drinking water (0.026 mg/ml) for 3 days. We performed metabolic profiling and determined the insulin sensitivity of adipose tissue and skeletal muscle tissues by in vitro incorporation of ^{14}C-U glucose. We isolated mRNA from liver and heart tissues and quantified the expression of candidate genes by real-time PCR (SYBR green assay on Cepheid Smartcycler II). Sequencing of cDNA of *Plzf* gene was performed using BigDye® Terminator v1.1 Cycle Sequencing Kit on ABI PRISM 310 Genetic Analyzer.
Results: The differential segment of PD/Cub origin in SHR-Lx PD5 congenic substrain spans approximately 1Mb between D8Rat43 and D8Rat94 markers with only 14 genes within the segment. In comparison with SHR, the SHR-*Lx* PD5 congenic strain displayed significantly lower total body weight both prior and after DEX challenge, lower relative adrenal weight, lower non-fasted triglyceride and total cholesterol concentrations. However, electrophoresis of lipoproteins suggested an unfavorable profile of increased LDL, VLDL and chylomicron and decreased HDL fractions. Both basal (81.8 ± 5.8 vs. 121.0 ± 12.8, p = 0.02) and insulin-stimulated (141.9 ± 9.9 vs. 226.0 ± 25.1 nmol glucose/g/2 hours, p = 0.01) incorporation of ^{14}C-U glucose into the glycogen of soleus muscle was significantly lower in SHR-*Lx* PD5 compared to SHR. The triglyceride content of the muscle tissue, the insulin sensitivity of adipose tissue as well as other metabolic parameters were not found to differ between the two strains. The expression of apolipoprotein A5 and hepatocyte nuclear factor 4 in liver was significantly increased in liver of SHR-*Lx* PD5. The sequence analysis of the *Plzf* transcription factor revealed 3 single nucleotide polymorphisms (SNPs), all in the first coding exon. Two SNPs are silent, but one leads to a threonine to serine substitution in SHR at amino-acid position 208 (T208S).
Conclusion: We have isolated a 1Mb genomic segment syntenic to human chromosome 11q23, which induces specific changes in body weight, lipid profile and related gene expression, overall glucose tolerance, and significant reduction in skeletal muscle insulin sensitivity as shown in SHR-*Lx* PD5 congenic substrain.
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A new variant in the promoter of the human Kv1.3 gene is associated with low insulin sensitivity and impaired glucose tolerance
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Background and Aims: The voltage-gated potassium channel Kv1.3 (*KCNA3*) is expressed in a variety of tissues including liver and skeletal muscle. In animal models, knock out of Kv1.3 has been found to improve insulin sensitivity and glucose tolerance. We, therefore, examined whether mutations in the Kv1.3 gene exist in humans and whether they are associated with alterations of glucose homeostasis.
Materials and Methods: We screened the promoter (2000 bp) and the coding region of the human Kv1.3 gene for mutations in 50 non-diabetic subjects by direct sequencing. Subsequently, all identified SNPs were analyzed in 552 non-diabetic subjects who participated in the ongoing Tübingen Family Study for type 2 diabetes. Of these, 304 had undergone a hyperinsulinaemic euglycaemic clamp.
Results: We identified 3 single nucleotide polymorphisms (SNPs) in the promoter region (A-845G, T-1645C and G-2069A, allelic frequency of the minor allele of 8, 41 and 16%, respectively). The -1645 C allele was associated with higher plasma glucose concentrations in the 2 hr OGTT (p=0.03) even after adjustment for sex, age and BMI (p=0.002). In addition, it was associated with lower insulin sensitivity (p=0.01, adjusted for sex, age and BMI). In contrast, SNP A-845G and SNP G-2069A were not associated with relevant metabolic parameters.
Conclusion: We show that a variant in the promoter of the Kv1.3 gene is associated with impaired glucose tolerance and lower insulin sensitivity. Therefore, the Kv1.3 channel may represent a candidate gene for type 2 diabetes.
This study was in part supported by a grant of the Deutsche Forschungsgemeinschaft DFG (KFO 114)

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The -174 G>C polymorphism in the promoter of the interleukin-6 gene is associated with total testosterone and insulin sensitivity in men
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Background and Aims: The pleiotropic cytokine interleukin-6 (IL-6) has many endocrine and metabolic effects. Recently, subcutaneous IL-6 administration was found to induce a suppression in testosterone concentrations in healthy men. However, there are few data on the influence of endogenous IL-6 on testosterone levels. We therefore studied (1) whether endogenous IL-6 levels are associated with testosterone levels and (2) whether the prevalent -174 G>C polymorphism in the IL-6 gene is associated with serum testosterone and insulin sensitivity.
Materials and Methods: 147 non-diabetic male subjects were metabolically phenotyped and genotyped for the -174 G>C polymorphism in the IL-6 gene.
Results: We found a significant negative correlation between serum testosterone and serum IL-6 levels (R square=0.08; p=0.008) We also found a positive correlation between serum testosterone levels and insulin sensitivity (R square=0.46; p=0.0001). Male carriers of the -174 C allele had lower serum testosterone levels (CX: 564 ± 20 ng/dl vs GG: 666 ± 25 ng/dl; p=0.01) and also had a lower insulin sensitivity compared to -174 G homozygotes (CX vs GG: 21.0 ± 1.3 vs 26.5 ± 1.8 arbitrary units; p=0.03). When analysing men and women together, the -174 G>C polymorphism tended to be associated with higher serum IL-6 levels (CX: 0.92 ± 0.12 ng/dl vs GG: 0.70 ± 0.08 ng/dl; p=0.06).
Conclusion: In conclusion, our results indicate that the -174 G>C polymorphism is associated with lower serum testosterone levels. This could be one of the mechanisms how this variant in the IL-6 gene influences insulin sensitivity in men.

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The Pro12Ala variant of the peroxisome proliferator-activated receptor gamma2 gene influences insulin sensitivity in healthy subjects participating in the RISC study
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Background and Aims: The Pro12Ala variant of the peroxisome proliferator-activated receptor γ2 gene *(PPARG)* has been shown to influence insulin sensitivity in man, with evidence that this is mediated through altered body composition. We examined the effect of the Pro12Ala variant on insulin sensitivity using data from the RISC (Relationship between Insulin Sensitivity and Cardiovascular disease) study.
Materials and Methods: Healthy subjects aged 30–60 yrs were recruited at 19 centres in 14 European countries. In addition to anthropometrics and lifestyle variables, each subject received a standard OGTT and euglycaemic hyperinsulinaemic (40 mU/m^2/min) clamp. We report here on the subjects who completed the baseline studies and for whom DNA was available for genotyping by RFLP assay.
Results: The study cohort consists of 1278 subjects (579 men and 699 women) aged 43.8 ± 8.4 yrs (mean±SD), with a mean BMI of 25.6 ± 4.0 kg/m^2. The allele frequencies were 0.89 and 0.11 for the Pro and Ala alle-

les, respectively, and they were in Hardy-Weinberg equilibrium. General linear model analysis showed a significant difference in insulin sensitivity between the genotypes (Pro/Pro vs. Pro/Ala vs. Ala/Ala: 54 ± 0.7 vs. 53 ± 1.2 vs. 67 ± 5.4 [mean±SEM] μmol/min/kg_{ffm}; p=0.04) after adjusting for age, sex, recruitment centre, waist circumference and BMI. Comparison of the Ala allele carriers [Pro/Ala+Ala/Ala] with the subjects homozygous for the Pro allele revealed no difference in insulin sensitivity. Conversely, subjects homozygous for the Ala allele (n=13) were more insulin sensitive (67 ± 5.4 vs. 54 ± 0.6 μmol/min/kg_{ffm}; p=0.014) compared to carriers of the Pro allele [Pro/Pro + Pro/Ala] after adjusting for the same factors and covariates. Subjects homozygous for the Ala allele also had lower adjusted fasting triglyceride levels (0.68(0.4–1.7) vs 0.95(0.3–7.4) [geometric mean(range)] mmol/l; p=0.01). However, this did not explain the greater insulin sensitivity which remained after including triglyceride levels as a covariate.
Conclusion: We confirm that the Pro12Ala *PPARG* variant influences insulin sensitivity in the healthy population. Specifically, subjects homozygous for the Ala allele are more insulin sensitive compared to the rest of the population, and this appears in part to be independent of differences in circulating triglyceride levels and measures of adiposity.
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Common polymorphisms of the adiponectin receptor genes as predictors of insulin resistance and type 2 diabetes: the STOP-NIDDM trial
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Background and Aims: Adiponectin is an adipose tissue specific protein having insulin-sensitizing and anti-atherogenic properties. Two adiponectin receptors, *AdipoR1* expressed in skeletal muscle, pancreas and liver, and *AdipoR2* expressed most abundantly in liver, are thought to mediate the effects of adiponectin.
We investigated whether single nucleotide polymorphisms (SNPs) of the adiponectin receptor genes predict the conversion from impaired glucose tolerance (IGT) to type 2 diabetes in the STOP-NIDDM trial.
Materials and Methods: The STOP-NIDDM trial, including 1 429 subjects with IGT, was a longitudinal, double blind, placebo-controlled randomised trial aiming to investigate the effect of acarbose compared to placebo on the prevention of type 2 diabetes. We genotyped 769 subjects whose DNA was available for the T/C polymorphism (rs:1539355) in intron 1 and the G/C polymorphism (rs:7539542) in the 3´UTR region of the *AdipoR1* gene, and for the T/G polymorphism (rs:1029629) in the promoter region of the *AdipoR2* gene using the TaqMan Allelic Discrimination Assays. Homeostasis model assessment for insulin resistance (HOMA IR) was calculated with the formula: fasting plasma glucose (mmol/l) * fasting serum insulin (mU/l) / 22.5.
Results: Subjects carrying the rare genotypes of SNPs of *AdipoR1* or *AdipoR2* converted to type 2 diabetes more often than subjects with the common genotypes, but the differences between the genotype groups were not statistically significant (p=0.447, p=0.708 and p=0.532, for the T/C and G/C polymorphisms of *AdipoR1* and the T/G polymorphism of *AdipoR2*, respectively). During the 3-year follow-up the T/C polymorphism of *AdipoR1* was associated with a significant change in fasting insulin level (TT genotype: -5.58 ± 49.7, TC genotype: -0.12 ± 62.3, CC genotype: 21.86 ± 71.7 pmol/l, p=0.004) and in HOMA-IR (TT genotype: -0.25 ± 2.5, TC genotype: 0.02 ± 3.3, CC genotype: 1.14 ± 3.5, p=0.003). Similar associations were observed in the placebo group (p=0.012 and p=0.007 for changes in fasting insulin level and HOMA-IR, respectively). The G/C polymorphism of *AdipoR1* did not show any association with insulin levels, but the rare genotype was associated with higher hepatic enzymes levels at baseline (p=0.010 and p=0.014 for AST and ALT, respectively). In the entire study population the rare genotype of the T/G polymorphism of *AdipoR2* was associated with an increase in fasting insulin level (TT genotype: -1.92 ± 54.0, TG genotype: -4.28 ± 58.2, GG genotype: 19.77 ± 69.2 pmol/l, p=0.045) and HOMA-IR (TT genotype: -0.06 ± 2.8, TG genotype: -0.20 ± 3.0, GG genotype: 1.04 ± 3.4, p=0.015).
Conclusion: SNPs of the adiponectin receptor genes did not influence on the conversion to type 2 diabetes in patients with IGT in the STOP-NIDDM trial. However, these SNPs were associated with elevated liver enzyme levels at baseline and with a significant change in fasting insulin level and HOMA-IR during the follow-up. Therefore, the adiponectin receptor genes may modify insulin sensitivity and predispose to type 2 diabetes.
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Abelson helper integration site-1 is associated with glucose and insulin levels in skeletal muscle
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Background and Aims: Exercise ameliorates insulin resistance in the polygenic obese diabetic animal model *Psammomys obesus*. In order to identify novel genes responsible for this improvement, the red gastrocnemius (RG) from chronically exercised, diabetic *P.obesus* was screened by microarray analyses. The Abelson helper integration site-1 (Ahi-1) gene was found to be significantly upregulated by exercise. Human Ahi-1 is located within a quantitative trait locus influencing metabolic syndrome related phenotypes in Mexican American and Finnish populations. The function of the Ahi-1 protein is currently unknown although it has recently been linked to Joubert syndrome. Ahi-1 contains an SH3 domain and seven WD40-repeats which are found in many signalling proteins. To further investigate its role in diabetes, Ahi-1 mRNA levels in lean, diabetic and obese diabetic *P.obesus* were quantitated and the associations between Ahi-1 polymorphisms and metabolic syndrome phenotypes were analysed in Mexican Americans.
Materials and Methods: Lean, insulin resistant and obese diabetic male animals (n=8 for each) were fasted for 24 h. Ahi-1 mRNA levels were quantitated using real time PCR. Bayesian quantitative trait nucleotide analysis was performed on 8 single nucleotide polymorphisms (SNPs) genotyped in 244 Mexican Americans. Statistical analysis was performed using ANOVA and t-test.
Results: In lean *P.obesus*, Ahi-1 gene expression was found to be predominantly expressed in skeletal muscle. Ahi-1 gene expression was upregulated by fasting in the RG of insulin resistant ($p<0.002$) and obese diabetic animals ($p<0.002$) and was positively correlated with plasma insulin ($p<0.03$) in these animals. Eight SNPs were typed in the Ahi-1 gene. One SNP was strongly associated with fasting glucose ($p<0.006$) while three others were nominally associated with fasting insulin ($p<0.03$).
Conclusion: Taken together, these data suggest a role for Ahi-1 in metabolism. Given the presence of signalling domains in the protein, current studies are focused on identifying proteins which interact with Ahi-1 in order to elucidate its functional significance in skeletal muscle metabolism.

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Resistin SNP-420 determines serum resistin levels in Japanese type 2 diabetic patients
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Background and Aims: Resistin, secreted from adipocytes, antagonizes insulin and causes insulin resistance in rodents. Recently, we reported that G/G genotype of a resistin gene promoter single nucleotide polymorphism (SNP) at -420 induces human type 2 diabetes (T2DM) susceptibility. Sp1/3 transcription factors specifically recognized the DNA element including -420G, and enhanced promoter activity, which could increase serum resistin levels. The objective of the study was to investigate effects of SNP-420 genotype and serum levels of resistin on clinical characteristics in Japanese T2DM patients and non-diabetic control subjects.
Material and Methods: We analyzed 493 T2DM and 563 control subjects including 96 new T2DM outpatients and 157 new control subjects in Ehime area in Japan. Genotypes were determined by PCR direct sequencing or the Taqman analysis. Fasting serum levels of resistin were measured by use of a human ELISA kit (LINCO Research). The clinical characteristics of these T2DM subjects and controls were analyzed by obtaining their informed consent. The study was approved by the ethics committee of the Ehime University Hospital and Ehime Prefectural Hospital.
Results: The onset of T2DM was ~4.5years earlier in T2DM subjects with G/G genotype than the others. When assessed in available samples of 198 cases and 157 controls, fasting serum resistin levels were significantly higher in T2DM (mean±SE, control 11.2 ± 0.5 vs T2DM 15.1 ± 0.7 ng/ml,

P<0.0001), and increased with the number of G alleles. Multiple regression analysis revealed that SNP-420 genotype was the strongest determinant for serum resistin levels both in cases and in controls (C/G; ~4.4ng/ml, G/G; ~10.6 ng/ml higher than C/C). The duration of T2DM, and HbA1c were also positively associated with these levels only in T2DM. Logistic regression analysis adjusted for age, gender, and maximum BMI revealed that serum resistin level was an independent factor for T2DM (P<0.0001).
Conclusion: The present study revealed that SNP-420 determines serum resistin levels in Japanese which could induce T2DM.

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Congenic WOKW.DA and DA.WOKW rats confirm quantitative trait loci for serum leptin on chromosome 16
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Background and Aims: WOKW rats develop a complete metabolic syndrome which is polygenetically inherited as shown by mapping of quantitative trait loci (QTLs) for single facets of the metabolic syndrome on different chromosomes in a cross of WOKW and disease-resistant DA rats. Beside others, a suggestive QTL for serum leptin (Lod score=3.5) was mapped on chromosome 16 in female (WOKWxDA)F2 hybrids whereas males showed no linkage. To confirm this QTL chromosome 16 congenic WOKW.DA (WOK.16D) and DA.WOKW rats (DA.16W) were generated, and genetically as well as phenotypically characterised.
Material and Methods: WOK.16D and DA.16W were generated by a cross of WOKW and DA rats using marker-aided selection. The resulting cross hybrids were repeatedly backcrossed with WOKW and DA rats, respectively using animals which were heterozygous at loci *D16Mit2*, *D16Mgh3* and *D16Mit16* and most homozygous for WOKW (WOK.16D) or DA (DA.16W) alleles at 180 background loci. After 5 backcross generations, the animals were intercrossed. Animals homozygous for WOKW or DA alleles at the loci on chromosome 16 were selected and founded the congenic WOK.16D and DA.16W rat strains. Founder animals were fine mapped with addition 30 polymorphic markers on chromosome 16. Twenty males and females of each strain were longitudinally characterised for body weight, blood glucose, serum lipids, leptin and insulin from the 8th to 32nd weeks of life. Rats were killed at 32 weeks, left and right inquinal adipose pads were removed and weighed to determine the adiposity index (AI).
Results: Despite selection on same markers in both congenics on chromosome 16, the fine mapping showed that DA alleles were found at 3 positions located between 9 and 11Mb, 19 and 35 as well as 40 and 73Mb in WOK.16D whereas WOKW alleles in DA.16W were found from 0.8 to 63Mb. The phenotypic analysis confirmed the QTL for serum leptin on chromosome 16 not only in females, but also in males. In congenic DA.16W serum leptin values showed a doubling in males and females (7.7 ± 2.0 and 3.0 ± 0.7ng/ml) compared to male and female DA rats (4.2 ± 1.0 and 1.2 ± 0.7ng/ml; p<0.0001). In addition, serum triglycerides and the adipose index (AI) were significantly increased in male and female DA.16W compared with parental DA rats. There was no effect in WOK.16D rats in serum leptin, but the AI of males was significantly lower and that of females significantly higher in WOK.16D rats compared with parental WOKW rats. Serum insulin was unaffected in DA.16W and significantly reduced in WOK.16D females compared with WOKW.
Conclusions: Because the leptin QTL was mapped around *D16Mgh3* and the chromosome was differently broken in WOK.16D and DA.16W rats, gene/s for serum leptin regulation and adiposity index must be located around 10Mb on chromosome 16 which is homologous with human 10q23. Sex-specificity could not be confirmed for serum leptin in DA.16W, but was found for AI in congenic WOK.16D, despite no QTL for AI was found in (WOKWxDA)F2 hybrids possibly reflecting a reversed sex-specific connection between serum leptin and AI.

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The prevalence of diabetes and the metabolic syndrome

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Prevalence of type 2 diabetes mellitus and the metabolic syndrome in Iceland 1967–2002
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Background and Aims: The prevalence of type 2 diabetes (T2DM) and the metabolic syndrome (MSX) is increasing at an alarming rate worldwide. The prevalence of T2DM in Iceland has been comparatively low and was thought not to be increasing before 1991. The aim of this study is to bring this information up to date by assessing the prevalence of T2DM and MSX in Iceland from 1967–2002 and to assess the effect of employing three different diagnostic criteria (WHO'85, ADA'97 and WHO'99) for T2DM and WHO'99 for MSX.
Materials and Methods: The data is based on data from the Icelandic Heart Association (IHA), mostly from a representative random sample of the Icelandic population (The Reykjavik Study) but also includes data from two other cohort studies from the the IHA. The total population of this survey is 16184, 7747 males and 8437 females, aged 45–64. The study period was divided into five equal size cross-sectional periods, each about 6 years: 1967–72, 1974–79, 1979–84, 1985–91 and 1997–02. The prevalence of T2DM and MSX was computed for every period.
Results: The age-standardized prevalence (95% CI) of type 2 diabetes in males, computed according to ADA'97 criteria has in a 30 year period risen from 3,3% (2,6–4,0) to 4,9% (3,5–5,3) which is an increase of 48%. In females the prevalence rose from 1,9% (1,4–2,4) to 2,9% (1,9–3,9) in the same period, a 53% increase. The time-trend was statistically significant for both males and females. For the years 1997–02 we estimated that for each individuals diagnosed with T2DM, there were 3 unrecognised cases. The prevalence of MSX also increased during the period but even more than the prevalence of T2DM. For males the increase was from 4,6% (3,8–5,4) to 8,7% (6,9–10,5), a 90% increase and for females the increase was from 2,8% (2,2-3,4) to 5,0% (3,8–6,2) which is an 80% increase.
Conclusion: The prevalence of T2DM and MSX is now increasing in Iceland as in the rest of the world although the increase has taken place somewhat later than elsewhere. This rise has parallelled the rise in obesity seen in Iceland during the same time period. The prevalence of T2DM is however still relatively low when compared to other western countries.

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Prevalence of metabolic syndrome and diabetes; association of risk factors among adults 25 years and above in urban Karachi
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Background and Aims: Several definitions of metabolic syndrome have been proposed in recent years. In Asians, obesity is less common and prevalence of metabolic abnormalities such as diabetes, hypertension and dyslipidemia are higher than Caucasians with similar BMIs. Thus the aim of this study was to estimate the prevalence of metabolic syndrome by ATP III, WHO, AACE and EGIR definitions in our population using standard and Asian cutoff for waist circumference and waist hip ratio as well as to evaluate the association of hs-CRP and insulin with obesity.
Materials and methods: A random selection of 500 households was done from the 85,520 households in an urban area of Karachi.
Socio- demographic information and anthropometric measurements were collected by field visits. At the end of the household visit, all adults ≥ 25 years were asked to undertake 8 hours fast for blood tests (Insulin levels, hs-CRP, blood glucose and lipid profile). A mid-stream urine specimen was also collected for pus cells, proteinuria and microalbuminurea.
Asian cutoffs for WC was taken as 85 cm for males and 80 cm for females while WHR was taken as 0.88 for males and 0.81 for females.
Results: Around 1493 subjects ≥ 25 years took part in the survey. Fifty one percent of these were females. Mean age was 40.1 ± 13.6 years. Mean BMI

was 24.5, WHR 0.91 and WC was 86.2 cm. Around 47% of subjects were hypertensive. Metabolic syndrome was assessed by four different definitions and by using modified obesity parameters for Asian population (Table1). Using kappa statistics, there was a moderate agreement between ATP and AACE definitions (K = 0.66). By using the modified Asian cutoffs for obesity we had an excellent agreement between ATP and AACE (K = 0.83).
Mean hs-CRP was 0.88 ± 0.94 mg/dl and mean Insulin levels were 8.1 ± 3.8 uIU/ml. Although hs-CRP was less in overweight/obese subjects compared to normal weight subjects while insulin levels were elevated in overweight/obese subjects, these were not statistically significant. According to FPG 5.2% were diabetic, 7.6% were impaired and 87.2% were normal. Normal Subjects had a mean hs-CRP and insulin levels of 0.83 ± 0.92 mg/dl and 7.98 ± 3.4uIU/ml, whereas subjects with glucose intolerance had mean hs-CRP and insulin levels of 0.83 ± 1.18 mg/dl and 7.36 ± 2.8uIU/ml respectively.
Conclusion: In our population large percentage of individuals are classified as having Metabolic Syndrome by ATP III and AACE definition. The prevalence of Metabolic Syndrome according to WHO and EGIR criteria is lower compared to the other two definitions. The prevalence increases by lowering the cutoffs of waist circumference and waist hip ratio.
Our study did not show a significant association between obesity and associated risk markers such as high insulin and hs-HS-CRP in this population.

Prevalence of metabolic syndrome according to four different definitions

		Standard criteria (%)	Modified criteria (%)
Adult Treatment Panel	(ATP-III)	19.4	26.2
American Association of Clinical Endocrinologist	(AACE)	32.1	32.1
World Health Organization	(WHO)	10.2	10.4
European Group for the Study of Insulin resistance	(EGIR)	6.7	7.5

Supported by Merck

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Prevalence of the metabolic syndrome in populations of Asian origin
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Background and Aims: To estimate the prevalence of the metabolic syndrome (MetS) and its individual components in populations of Asian origin using the NCEP ATPIII (National Cholesterol Education Program Adult Treatment Expert Panel III) definition.
Materials and Methods: The age-and sex-specific prevalences of the MetS were estimated in 6750 men and 7824 women without diabetes aged 25–74 years in 10 DECODA study cohorts of Asian origin, including native and Mauritian Indians, native and immigrant Chinese, and native Japanese.
Results: Prevalence of the MetS in Indians living in urban area of India was as high as that in Mauritian Indians, but higher than in urban Chinese and in rural Japanese (Table). The prevalence increased with age up to 55–64 or 65–74 years in women, while in men, the trend varied by population. Men tended to have a higher prevalence than women at age of 44 years or younger, but lower at older ages. The prevalence of hypertension was less variant among the different ethnic groups (38.9% to 41.0% in men, and 30.5% to 34.2% in women). Central obesity by the ATP III criteria was uncommon in men (0.7% to 3.2%), but more frequent in women (7.4% to 22.8%). High triglycerides were more frequent in men (25.2% to 33.7%) than in women (12.7% to 20.1%). The prevalence of low high-density lipoprotein cholesterol was extremely higher in urban Indians (63.1% in men, and 76.1% in women) than in other populations (ranged from 14.2% to 31.9% in men and 25.0% to 52.4% in women). The prevalence of impaired fasting glucose was 11.7% in Mauritian Indian men but lower than 10% in all other ethnic groups. Among subjects with the MetS, 83.7% to 95.0% of the men and 80.2% to 92.4% of the women had hypertension. High triglycerides was more common in males (87.4% to 100.0%) than in female with MetS (61.6% to 75.9%), but females with MetS had a higher prevalence of central obesity ([49.4% to 69.1%] vs. [3.1% to 26.1%]).
Conclusion: The prevalence of the NCEP ATPIII MetS was higher in Indians than in Chinese and Japanese subjects. The phenotypes of individuals with the MetS differed between men and women and between ethnic groups with regard to differences in individual components included in the definition. Hypertension was the most common factor that was associated with a full diagnosis of MetS in these Asian populations.

Prevalence (%) of the metabolic syndrome in four populations of Asian origin

Age	Mauritian Indian		Native Indian (Urban)		Chinese (Urban)		Japanese (Rural)	
	Men	Women	Men	Women	Men	Women	Men	Women
25–34	7.1	3.9	11.5	3.5	4.2	1.4	–	–
35–44	10.6	8.9	12.6	12.4	7.0	3.7	7.8	1.4
45–54	13.2	17.2	16.3	22.3	8.1	13.2	4.3	4.8
55–64	12.6	26.0	13.8	31.5	11.2	23.8	4.1	9.5
65–74	12.1	27.1	12.1	26.2	15.5	23.4	1.7	10.8
All age standardized (95% CI)	10.7 (9.4–11.9)	13.9 (12.6–15.2)	13.2 (10.5–16.0)	16.5 (13.8–19.2)	8.0 (7.0–9.0)	10.3 (9.3–11.4)	5.1 (3.5–6.6)	5.6 (4.2–6.9)

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Prevalence of carbohydrate disorders and arterial hypertension in representative sample of adults in Poland. Results of NATPOL PLUS Survey
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Background and Aims: There was a lack of national data concerning the prevalence of main cardiovascular (CV) risk factors in adult population (29,7 mln) in Poland.
Materials and Methods: In year 2002, a representative sample of 3051 adults, randomly recruited from 304 geographical places, was examined in the NATPOL PLUS Survey. The main aim of this first nationwide, cross-sectional survey was to assess the prevalence and control of all main cardiovascular risk factors. Blood pressure and anthropometrical measurements were performed in all randomly selected subjects (age range 18–94 years); 76,5% of them (n=2333) also completed laboratory tests (fasting glycaemia, insulin, lipids, hs-CRP). In the present study data on carbohydrate and blood pressure disorders are presented. Arterial hypertension was defined as SBP≥140 mmHg and/or DBP≥90 mmHg on 3 separate visits or antihypertensive medication. Diabetes was assessed by two separate analyses and defined as fasting glucose level ≥126 mg/dl or hypoglycaemic medication. Classification of blood pressure and carbohydrate disorders was based on ESH and EASD standards, respectively.
Results: Arterial hypertension was found in 29% of respondents, high normal, normal and optimal blood pressure in 30%, 21%, and 20% respectively. Hypertension was treated efficiently (<140/90 mmHg) in 12% of cases. Diabetes was found in 5,6% of all respondents; 16% of them were not aware of disease. IGT was found in 0,6% and IFG in 1,5% of examined subjects. Mean fasting glucose level increased with age. It was equal to 81,7 mg/dl in subjects aged between 18 to 30, and 95,6 mg/dl in subjects over 75 years of age. In a subgroup of people above 50 years of age the prevalence of diabetes was equal to 12,9%, IGT - 0,8% and IFG - 1,7%. Arterial hypertension was found in 72% of all subjects with diabetes.
Conclusion: The data of NATPOL PLUS Survey, the first nationwide, representative study in Poland, showed that according to present European guidelines there is 8,6 mln of subjects with arterial hypertension and 1,65 mln with diabetes. These figures, in concert with poor detectability and control of hypertension and diabetes, call for urgent preventive measures, which should also be targeted in „prehypertension" and „prediabetes" subpopulations.

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The metabolic syndrome in university students with family history of diabetes mellitus - a preliminary report of the University Smoking and Obesity Assessment Program (University SOAP)

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Background and Aims: In order to evaluate the impact of the family history of diabetes on the healthy and young university students in Taiwan, students were randomly recruited from 2 universities under the University SOAP.
Materials and Methods: From Sept. 2003 to Apr. 2004, 656 students were enrolled. All the participants were measured BMI, blood pressure, waist and hip circumferences and other biochemical variables including fasting serum glucose, TG, cholesterol, HDL-Chol, LDL-Chol, uric acid, renal and liver functions. Meanwhile, family history of diabetes, hypertension, dyslipidemia, obesity, coronary artery disease (CAD), and cerebral vascular accident (CVA) were collected. Personal habitudes of smoking and physical activity were also recorded. The descriptive statistics and logistic regression were used for data analysis.
Results: According to the database of the University SOAP, 209 cases had diabetes in their family and only 274 cases without any family history. The incidence of different co-morbidities occurring in students with family history was significantly increased in BMI ($p = 0.000$), diastolic blood pressure ($p = 0.024$), systolic and/or diastolic blood pressures ($p = 0.029$) and any one of the co-morbidities as a group ($p = 0.012$) as compared with students without family history. By using the gender approach, the results were interestingly disclosed that the male students had significantly higher odds of having co-morbidities of BMI ($p = 0.000$), diastolic blood pressure ($p = 0.015$), systolic and / or diastolic blood pressures ($p = 0.011$), HDL-Chol ($p = 0.043$) and any one of the co-morbidities as a group ($p = 0.004$) in diabetes family, whereas, no statistical difference in term of incidence of co-morbidities was found in female students with or without family history. Furthermore, metabolic syndrome in this student cohort, the result was demonstrated that metabolic syndrome ($p = 0.007$) was significantly higher in odds ratio in students with family history. Again, by gender analysis, only male student had significantly higher odds of having metabolic syndrome ($p = 0.011$) in family history group.
Conclusion: These results clearly indicated that the family history of diabetes has already had anthropometric and metabolic impacts on the young, active and otherwise healthy offspring of next generation at the university ages in all aspects of the co-morbidities of diabetes and metabolic syndrome. The university female gender, however, seemed to be spared at that age. The intervention program for early primary prevention of this insidious developing insulin resistance syndrome would be remaining a great challenge.

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Prevalence of the metabolic syndrome in a Canarian community according to the WHO (1998) and ATP-III (2001) criteria. Impact of the proposed modifications on the ATP-III criteria on the prevalence

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Background and Aims: The metabolic syndrome is an entity of growing clinical relevance, and its prevalence seems to be very high in our community. However, an internationally accepted definition has not been reached. Our objective in this work is to assess the prevalence of the metabolic syndrome in a Canarian community (Santa María de Guía) and its variation according to the chosen criteria, including two recently proposed modifications on the NCEP ATP-III criteria.
Materials and Methods: The data (anamnesis, anthropometry and analyses) were obtained from the database of a population survey (The Guía Study), from a total of 666 subjects (30 years old at least), chosen at random from the municipal roster (after stratification by age and sex). The data recollection included a standard OGTT in all except the known diabetic subjects. The proposed changes in the first NCEP ATP-III criterium (fasting glucose ≥ 110 mg/dl or known diabetes) are: 1) including the subjects with impaired glucose tolerance, and 2) lowering to 100 mg/dl the fasting glucose threshold.
Results: The observed prevalence of the metabolic syndrome was 45.2% (WHO criteria, 37.4% adjusted to the world population), and was significantly higher in older subjects (27.5%, 51.6% and 62.4% in 30–49, 50–69 and 70+ years, $p = 0.000$); with the original NCEP ATP-III it was 44.3% (37.1% adjusted), with similar changes with the age (28.7, 50.8 and 58.2%, $p = 0.000$). Including the subjects with impaired fasting glucose, the prevalence rose to 51.2%, adjusted 47.4% (31.9, 59.6 and 67.9%, $p = 0.000$), on the other hand, lowering to 100 mg/dl the fasting glucose threshold the prevalence rose to 55.6%, adjusted 42.7 (37.8, 62.0 and 72.7%, $p = 0.000$). The kappa concordance index was 0.86 for the WHO and the original NCEP ATP-III criteria; 91.4 of the WHO positive subjects were NCEP ATP-III positive, and 93.2% the other way around, but the concordance was poor when the first NCEP-ATP III criterium was modified.
Conclusion: The prevalence of the metabolic syndrome in our studied community is very high; the WHO and NECP-ATP-III criteria yield similar prevalences, but both proposed modifications to the first NECP ATP-III criterium yield substantially increased prevalences.

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Prevalence of impaired glucose regulation (2003 modified ADA criteria) in Telde (Canary Islands) (Spain). Differences in cardiovascular risk factors, insulin resistance and insulin secretion

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Background and Aims: In 2003 the ADA modified the 1997 criteria for the diagnosis of impaired fasting glucose with a lower cut point of 5.55 mmol/l. We studied the prevalence of IFG,IGT and combined IFG/IGT in a adult Canarian population and compared the cardiovascular risk profiles, prevalence of metabolic syndrome and degree of insulin resistance and insulin secretion in the normal population and in the individuals with different categories of impaired glucose regulation.
Materials and Methods: 1030 subjects aged 30–82 years were included in the study.They were recruited from a cross-sectional population study on diabetes and cardiovascular risk factors.Participants completed a survey questionnaired and underwent blood pressure measurements,anthropometry,blood samples, and a 75-g standardized oral glucose tolerance test. The categories of glucose homeostasis were defined based on modified ADA criteria 2003. Risk factors for cardiovascular disease and features of metabolic syndrome (blood pressure, waist circumference, BMI, insulin, lipids, CRP, homocystein, PAI-1, von Willebrand Factor, fibrinogen) were analysed. The HOMA and MacAuleys and QUICKY indixes were used for the evaluation of beta-cell function and insulin resistance.
Results: After excluding those with diabetes 902 subjects participated in the study, 663 (73,5%) had normal glucose tolerance(NGT), 132 (14,6%) had isolated IFG, 59 (6,5%) isolated IGT, and 48 (5,3%) combined IFG/IGT. The normal population had the lowest cardiovascular risk and lowest prevalence of metabolic syndrome. There was not differences between IFG and IGT in cardiovascular risk factors but IGT had a significant higher prevalence of metabolic syndrome. The combined IFG/IGT had the highest cardiovascular risk profile and highest prevalence of metabolic syndrome. We didn`t found differences between IFG and IGT in insulin resistance but the IFG group showed to have impaired insulin secretion.
Conclusion: With the new ADA criteria and as it was expected we observed a high prevalence of IFG. Individuals with impaired glucose regulation have an increased cardiovascular risk specially those with combined IFG/IGT. In our study isolated IFG and isolated IGT had similar CVD risk profiles and similar degree of insulin resistance but the prevalence of metabolic syndrome was higher in IGT subjects wich can explain because IGT is more strongly associated with CVD outcomes.

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Prevalence of type 2 diabetes mellitus, hypertension, prehypertension and metabolic syndrome in a high socioeconomic population of a north Indian city

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Background and Aims: To study the prevalence of type 2 diabetes mellitus, hypertension, prehypertension and metabolic syndrome in an affluent community of north India
Materials and Methods: An urban colony of high income group in Lucknow, containing 700 families was selected for this study. All the subjects aged 30 years and above were invited to participate in the study. All subjects except known diabetics underwent oral glucose tolerance test according to WHO (1999) guidelines. The time interval between fasting and 2 hr post glucose/postprandial blood collection was utilized for obtaining the history and anthropometric measurements.
Results: Of 1746 eligible subjects (>30yrs) in this community, 1112 subjects participated in the study. The response rate was 63.6%.
Prevalence of diabetes was significantly higher in men (28.3% VS 20.7%). Of the total no. of diabetic subjects, 62.2% were known diabetics. Prevalence of diabetes increased with age and a maximum prevalence was seen in the age group 70 and above (38.4%). In contrast, prevalence of IGT was maximum in the age group 30 to 39 (22.1%). Prevalence of diabetes was significantly higher in subjects with family history of diabetes (33.5%) than in those without family history (18.4%) ($P<0.05$). The prevalence of hypertension was present in 38.6% of study population. Hypertension was significantly higher in men (42.9%) as compared to women (34.2%). Of the subjects with hypertension, 27.6% were previously known to have the illness and 11.0% patients were diagnosed hypertension for first time. The prevalence of hypertension increased with age in both men and women. The prevalence of hypertension in the youngest age group (30 years to 39 years) is 13.7%. In our study population there is high prevalence of prehypertension and hypertension. Approximately 68.2% of study populations had prehypertension or hypertension. Even in young age group of 30 to 39 yrs, the 50% study population is having either hypertension or prehypertension. Approximately 72% of overweight individuals and 83% of obese participated had prehypertension and hypertension, where as the prevalence was only 58% in the normal weight group. In study population 80% were overweight (BMI ≥23 kg/m^2), while central obesity (waist circumference >85 cm in males and >80 cm in females, Indian criteria) was found in 84% population surveyed. The most frequent lipid abnormalities were hypertriglyceridemia (35%) and low HDL (54%); elevated LDL (>130 mg/dl) was present in 18% of males and 29% of females surveyed. The prevalence of metabolic syndrome (NCEP-ATP I I I criteria) was 33%. Diabetes and IGT were tested as dependent variables separately. Age more than 40 years, male gender, family history of diabetes, BMI and high WHR showed significant association with diabetes.
Univariate regression analysis also revealed age, sex, BMI, waist, S. cholesterol, S. triglyceride, glucose intolerance, and family history hypertension to be associated with hypertension.
Conclusion: Prevalence of diabetes mellitus, hypertension, prehypertension and metabolic syndrome is very high in high socio-economic population as compare to reported earlier in the mixed population. And in short period of time there will huge burden of these diseases on Indian economy, as we are already suffering from lack of resources, awareness and facilities for management.

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An epidemiologic study on the prevalence of diabetes mellitus, glucose intolerance and metabolic syndrome in the adult population in the Republic of Cyprus

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Background and Aims: Aim of this study was to measure the prevalence of Diabetes Mellitus (DM), IGT and IFG as well as the prevalence of Metabolic Syndrome and its components in the population of Cyprus.
Materials and Methods: A representative sample of 1200 individuals (580 men and 620 women) aged 20–80 participated in this study, selected from the 2003 electoral list using the random stratified method, considering age, sex, socio-economic status and rural or urban residence. All subjects were studied after an overnight fast. An OGTT, WHO criteria, was done, blood was taken for lipid determination, blood pressure, height, weight and waist circumference were measured with standard methodologies. IFG was defined according to the ADA criteria; "New" IFG according to the lower cut-off point of fasting plasma glucose i. e. 100 mg% and the Metabolic Syndrome was diagnosed by the NCEP ATP criteria.
Results: The prevalence of various categories of glucose intolerance and the Metabolic Syndrome are presented in the Table.

N	1200	580 men	620 women
Known Diabetes	6.5%	8.5%	4.6%
OGTT Diagnosed Diabetes	3.8%	5.9%	1.8%
Total Diabetes	10.3%	14.4%	6.4%
Impaired Glucose Tolerance	6.5%	6.3%	6.6%
Impaired Fasting Glucose / "New" IFG	4.4/17.4%	4.6/18.8%	4.2/16.0%
1. Abdominal Obesity (Male >102 cm, Female >88 cm)	34.8%	32.1%	37.4%
2. Triglycerides > 150 mg/dl	24.3%	33.6%	15.5%
3. Blood Pressure ≥130/85 mmHg	21.2%	23.4%	19.1%
4. Fasting Glucose ≥110 mg/dl	12.4%	16.2%	8.8%
5. HDL Cholesterol (Male<40 mg/dl, Female <50 mg/dl)	34.8%	39.2%	30.7%
Prevalence of Metabolic Syndrome (3 or more of the above 5 factors)	19.5%	23.2%	16.0%

The prevalence of known diabetes is among the highest in Europe, compared to five centers of the DECODE study with similar sex and age distribution, 6.5% versus 2.7, 4.0, 4.3, 6.4, 8.0 and higher than the NHANES, 6.5 vs 5.8%. The same is true for OGTT diagnosed diabetes, 3.8% versus 1.9, 2.5, 3.5, 3.7 and 6.4% in the DECODE study and 3.8vs2.4% in the NHANES.
Conclusion: The prevalence of both known and OGTT diagnosed diabetes in Cyprus is among the highest in Europe and USA, while the prevalence of the Metabolic Syndrome is not higher than in Europe or USA. To every two known diabetics corresponds one undiagnosed, suggesting that programmes for early detection of diabetes are needed in Cyprus.

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Levels of glucose and lipids in a European population with diabetes

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Backgrounds and Aims: Individuals with type 2 diabetes have a 2–4 fold increase in risk of dying from cardiovascular diseases. This is mainly due to the fact that these individuals have an unfavourable cardiovascular risk profile including dyslipidaemia. Because of the additive cardiovascular risk of hyperglycemia and dyslipidaemia, lipid abnormalities should be detected and treated as a part of comprehensive diabetes care. Although there is a high risk associated with dyslipidemia and a strong recommendation for treating patients with lipid lowering agents, there are very few current data from large population-based studies referring to the prevalence of lipid disorders in type 2 diabetes patients. The aim of this study is to asses the proportion of the population with diabetes in Europe qualifying for concomitant treatment of uncontrolled hyperglycemia and dyslipidaemia according to the European guidelines on CVD prevention.
Materials and Methods: The analyses were based on data from the DETECT-2 project, an international data pooling collaboration of population based surveys covering all major inhabited continents. This analysis included 14,352 individuals from Europe (North-, Central- and South Europe) in the age range 25–95 year and examined between 1995 and 2002. Of these, 1,514 were type 2 diabetics. Not all individuals had information of all the components investigated.
Results: The analyses show that according to the European guidelines for treatment goals in individuals with type 2 diabetes, more than 67% have an HbAlc > 6.1% in Northern Europe and more than 85% of the population with diabetes have dyslipidemia (highest in South Europe with 92%).

	Europe North	Europe Central	Europe South	Europe Total
Individuals with DM	998 (7.0)	400 (14.2)	116 (18.2)	1514 (10.6)
Dyslipidemia	85.5 (83.0–87.8)	N.A.	92.1 (85.5–96.3)	836, 86.3 (83.9–88.4)
Total cholesterol > 175 mg/dl	83.2 (80.7–85.6)	95.5 (93.0–97.3)	91.3 (84.6–95.8)	1257, 87.3 (85.5–89.0)
LDL cholesterol ≥100 mg/dl	87.5 (85.1–89.6)	N.A.	92.1 (85.5–96.3)	853, 88.0 (85.8–90.0)
HDL <40 mg/dl (M) or < 46 mg/dl(F)	37.4 (34.2–40.8)	N.A.	44.3 (35.1–53.9)	371, 38.2 (35.2–41.4)
Triglycerides >150 mg/dl	51.7 (48.3–55.0)	N.A.	43.9 (34.6–53.5)	515, 50.8 (47.7–53.9)
HbA1c > 6.1%	67.2 (62.3–71.7)	N.A.	N.A.	270, 67.2 (62.3–71.7)

The thresholds for HDL and triglycerides are markers of increased cardiovascular risk rather than treatment goals. Values are proportions (%) and (95%-CI). For Europe total, the number of individuals with diabetes and the given condition is included as well in each cell. The crude number of individuals with diabetes is listed in the second row (prevalence of diabetes, %).

Conclusion: A very large proportion of the individuals with diabetes in Europe qualify for treatment of hyperglycemia and dyslipidaemia according to the European guidelines on CVD prevention.
Support: Bristol-Myers Squibb

PS 18

Differentation of stem cells into insulin expressing cells

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Cell therapy of diabetes with insulin-producing cells derived from adult stem cells
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Background and Aims: The presence of stem cells in the adult pancreas which can give rise to beta cells is controversial discussed. The aim of this study was to isolate adult pancreatic stem cells (APSCs) from mouse pancreas and develop strategies for the differentiation into insulin-producing cells for cell therapy of diabetic mice.
Materials and Methods: Cell lines were obtained from adult pancreas, cloned by limiting dilution and analysed by RT-PCR and FACS for the expression of stem cell and beta-cell progenitor markers. Cell maturation was induced by changing the culture conditions (low-glucose, low serum, growth factors). Differentiated APSCs, marked with EGFP by lentiviral transduction, were transplanted under the kidney capsule of streptozotozin-diabetic C57/Bl6 mice (blood glucose levels > 400 mg/dl).
Results: We isolated a novel cell population from adult pancreas which have a high self-renewal capacity, express stem cell associated markers (e. g. nestin, BCRP1, sox1), in some lines beta-cell progenitor markers (pdx1, isl1, and CK19, respectively), but no markers of mesenchymal or hemopoietic stem cells (CD73, CD90, CD105, CD34, CD45, CD117). After differentiation expression of proinsulin was observed. Transplantation in diabetic mice leads to a significant reduction of the blood glucose levels (< 200 mg/dl).
Conclusion: Beta-cell progenitor cells can be isolated and expanded from adult pancreas. Transplantation experiments revealed that these cells produce insulin in vivo and improve blood glucose levels in diabetic mice. The APSCs represent a novel pancreatic progenitor cell population and are promising candidates to establish a cell replacement therapy for diabetes mellitus.

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A preliminary study on differentiation of the bone marrow-derived stem cells into insulin-producing cells
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Backgrounds and aims: Cell-based therapy is an approach that could potentially move us towards a cure for diabetes and finding new sources of insulin-producing cell is an intense quest. It has been appreciated that bone marrow, not only as a distributor of hemopoietic progenitor, but also as a systemic supplier of progenitors that have the potential to be differentiated into special cells and join in the repair of solid organs. Dr. Verfaillie found the versatile cells, called multipotent adult progenitor cells in the bone marrow (MAPC), has higher potential of multipotency and self-renewing ability. We investigated if it could be induced into insulin-producing cells in vitro and contribute to islet regeneration in vivo.
Methods: Cells were cultured under the conditions referred by Dr. Verfaillie with some modifications. When cultured on high glucose medium with GLP-1 and nicotinamide, the expression of genes during the development of islets were detected by mRNA and insulin immunochemistry. Female Balb/c mice were used as recipients, while male Balb/c mice with 3-week-old as donors. Diabetic mice induced by streptozocin(STZ) received $1\sim2\times10^6$ carboxyflurescein diacetate,succinmidyl ester(CFSE, a kind of fluorescent label) labeled cells via tail vein injection within 24 hours of irradiation. At the end of the 4-week test, chromosome Y by PCR in these female mice was detected and Cy3 labeled insulin and CFSE stain were observed under under fluorenscent microscope. Pancreas were harvested for insulin immunochemistry and insulin positive area were calculated. Results were presented as Mean±SD and positive or negative. Data were analyzed with student's unpaired t test(two tail). A P level less than 0.05 was considered significant.
Results: The cells expressed ngn3 and pdx-1 at early stage(day 7) of inducing with nkx2.2,pdx-1,ins-2 expressed at late stage(day 14), and at day 14, the cells were 5.6 ± 1.4% insulin positive; Chromosome Y by PCR in these female mice was detected in all of them. Cells stained with CFSE and double stained with CFSE and insulin were found in islets with the total number per section of the former being 3.5 ± 2.4,the latter 1.2 ± 1.1 and the percent-

age of double stained cells to the total number of both kinds of cells summed up to 24.3 ± 17.4%, suggesting that this kind of BMDSC could home at the diabetic pancreatic site with a portion took the phenotype of insulin positive cells. Insulin immunochemistry found that the islet neogenesis increased with the insulin positive area at 100× magnification under microscope per section higher in these mice when compared with those without transplantation (474.13 ± 380.01 vs 237.45 ± 756.78 μm^2, p=0.037).Although there was no significant attenuation of blood glucose, the surviving time of the mice increased.
Conclusions: This kind of bone marrow-derived stem cells (BMDSC) had the possibility to be induced into insulin-producing cells in vitro. In vivo, the transplanted cells could home at the pancreatic site of STZ-induced diabetic mice and contribute to islet regeneration.

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Comparison of three cell culture protocols in differentiation of embryonic stem cells into insulin-secreting cells
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Background and Aims: To drive differentiation of embryonic stem cells (ES cells) *in vitro*, three cell culture protocols, that is embryonic body (EB) formation, EB formation-monolayer and monolayer, have been developed so far. However, it remains unknown whether there are any different effects on the differentiation of ES cells among the three cell culture protocols. The aim of the present study is to compare the effects on differentiation of mouse ES cells into insulin-secreting cells among the three cell culture protocols.
Materials and Methods: E14.1 mouse ES cells were treated by GLP-1, betacellulin, activin A, bFGF and nicotinamide using EB formation, EB formation-monolayer (for the first 16 days ES cells were cultured via EB formation, on day 17, the EBs were dissociated into single cells, then the cells were cultured in monolayer) and monolayer culture protocols for 30 days. On day 30, mRNAs of insulin, glucagon, somatostatin, PP, PDX-1, Beta2 and Ngn-3 were investigated using RT-PCR, insulin expression was also examined by DTZ-staining and immunohistochemistry. The percentage of insulin-secreting cells was evaluated by flowcytometry.
Results: mRNAs of insulin were detected in the EB formation group, EB formation-monolayer group and monolayer group, but mRNA of insulin was strongest in the EB formation-monolayer group and the weakest in the monolayer group. In addition, mRNAs of glucagon, somatostatin, PP, PDX-1, Beta2 and Ngn3 were detected in the EB formation group and EB formation-monolayer group, all except PP and Beta2 were detected in the monolayer group, DTZ-staining positive cells and insulin immunohistochemical staining positive cells were observed in all the three groups. The percentage of insulin-positive cells of the differentiated cells in the EB formation-monolayer group was higher than that in the EB formation group (17.8%± 3.6% *vs* 13.6%± 3.7%, n=6, $P<0.01$), and the EB formation group was higher than the monolayer group (13.6%± 3.7% *vs* 9.3%± 1.6%, n=6, $P<0.01$).
Conclusion: Among the three cell culture protocols, EB formation-monolayer is the most effective approach in the induction of mouse ES cells to differentiate into insulin-secreting cells *in vitro*.

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Comparative analysis of gene expression and morphology of mouse embryonic stem cells differentiated towards insulin-producing cells using four different cell culture protocols
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Background and Aims: The generation of insulin-producing cells from mouse embryonic stem cells (mESC) has been the subject of several studies. However, controversial results were obtained. Thus the aim of this study was to compare the effect of four distinct differentiation protocols on the gene expression and the morphology of mESC.
Materials and Methods: Mouse embryonic stem cells from the D3 cell line were differentiated according to four different protocols using a combination of serum-free or fetal calf serum (FCS) supplemented media. In addition, in one protocol, the cells were cultured in spinner flasks under constant stirring. To assess the effect of these culture techniques the expression of marker genes was examined using *real-time* RT-PCR. In parallel the morphology of the cells was monitored by electron microscopy (EM).
Results: The results obtained when the cells were differentiated according to a serum-free protocol show that the cells express marker genes for endocrine differentiation. Morphological analyses revealed that roughly 20% of all cells were differentiated. In addition 30–40% of the cells were prone to cell death by apoptosis (two thirds) and necrosis (one third), respectively. When the cells were instead exposed to media supplemented with 5% FCS in the final stage of the protocol insulin gene expression decreased by around 50%. The same decrease could also be shown for glucagon and somatostatin. But the expression of Glut-2, Sur-1, Kir6.2, Nestin and Nkx6.1 could benefit from FCS supplementation. Moreover, a significant fraction of cells with endocrine characteristics and a higher degree of differentiation were identified in EM. Nonetheless the proportion of cells which undergo apoptosis was kept unaffected. However, when the step of selection of nestin positive cells was removed from the protocol and the cells were maintained for 12–14 days in serum-free medium and subsequently in medium containing 5% FCS the insulin expression was normalized again. The expression of other beta cell marker genes was also significantly improved. Again EM displayed a higher differentiation status of the cells. When mESC were cultured in suspension in a spinner culture protocol the expression of all genes besides Oct-4 was significantly decreased. The cells showed a well preserved embryonic phenotype in EM with no signs of differentiation. The high level of Oct-4 expression was confirmed also by the expression of a second embryonic marker gene, ERas.
Conclusion: Here we report that utilisation of different culture conditions affects embryonic stem cells with respect to their differentiation towards insulin-producing cells. We were able to establish a new differentiation protocol, which, at the same time, was able to induce insulin gene expression as well as of beta cell specific genes in cells with morphological beta cell characteristics and at the same time to reduce the rate of apoptosis. The usage of spinner conditions, on the other and, maintained stem cells in an undifferentiated state. We therefore conclude that proper conditions are required for differentiation and maturation of mESC into insulin-producing cells.

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Activation of the beta-cell genetic program in proliferating M-CSF-treated human blood monocytes
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Background and Aims: The shortage of human islets hampers islet transplantation for treatment of type I diabetes. The exploration of alternative sources to generate surrogate beta-cells is therefore crucial. To date, insulin-containing cells have been derived from stem cells using either defined culture conditions or ectopic expression of key pancreatic endocrine transcription factors (TF). Despite progress, the ideal surrogate beta-cell has not been generated indicating that steps in differentiation are still poorly understood. Recently, human peripheral blood monocytes treated with macrophage-colony stimulating factor (M-CSF) were shown to acquire a pluripotent phenotype that can be differentiated into neuronal-, epithelial- or hepatic-like cells. Our aim was to initiate the beta-cell genetic program in M-CSF-treated human monocytes by ectopic expression of pancreatic endocrine TFs.
Materials and Methods: Human monocytes were isolated from blood of normal donors using standard protocols and cultured in the presence of M-CSF. Proliferation was assessed by BrdU assay up to 28 days. Cells were infected with adenoviruses expressing mNgn3, mNeuroD or mPax4 and cultured for an additional 2 and 4 days. RNA was isolated and endogenous transcript levels for IPF1, hIsl1, hNeuroD, hPax4 and hNkx6.1 were determined by quantitative RT-PCR.
Results: Monocytes cultured in the presence of M-CSF acquired an elongated phenotype within 5 to 8 days whereas untreated cells remained round and were slowly dying. BrdU incorporation revealed that more than 20% of M-CSF-treated cells were actively replicating up to 12 days. Furthermore, treatment with hepatocyte growth factor (HGF) induced the foetal liver marker α-foetoprotein confirming the pluripotent phenotype of the cells. We next evaluated the impact of forced expression of mNgn3, mNeuroD or mPax4 on activation of endogenous levels of IPF1, hIsl1, hNeuroD, hPax4 and hNkx6.1, all absent in control non-infected cells. Mouse Ngn3 induced endogenous levels by approximatively 53% for hPax4, 22% for hNkx6.1, 15% for IPF1 and 12% for hIsl1 and hNeuroD as compared to endogenous human islet levels. Ectopic expression of mNeuroD mainly stimulated endogenous hPax4 and hNkx6.1 levels (29 and 20%, respectively) whereas IPF1, hIsl1 and hNeuroD expression was ~14%. Mouse Pax4 evoked endogenous hPax4 levels similar to those found in human islets whereas IPF1 was induced by 19%, hIsl1, hNeuroD and hNkx6.1 by 13%. Insulin

transcript was not detected under these conditions reflecting insufficient TF expression. As a control, HeLa cells did not respond to forced expression. Insulin and endogenous TF mRNAs were detected only when non-infected M-CSF-treated monocytes were cultured in a defined medium.
Conclusion: These results show that human monocytes can be programmed to express beta-cell markers. Overexpression of single TF was not sufficient to induce insulin mRNA indicating the need for orchestrated activation of multiple TFs for differentiation towards the pancreatic endocrine phenotype

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Coordinated activation of pancreatic developmental genes during differentiation of human bone marrow-derived stem cells into insulin, amylin, glucagon and somatostatin expressing cells
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Introduction: Stem / progenitor cells with the potential to generate insulin expressing cells have been identified in several organs including the pancreas, liver and bone marrow. Here we describe a coordinated activation of pancreatic developmental genes and transcription factors during formation of islet like clusters from human bone marrow- derived stem cells.
Material and Methods: Human bone marrow derived mesenchymal stem cells (hMSC) were isolated and expanded in DMEM with 10% FBS and FGF. For induction of differentiation, hMSC were cultured for three days in serum free differentiation medium (DMEM/F12) supplemented with nicotinamide, activin-A and exendin-4 hepatocyte growth factor and pentagastrin. On days 0, 1, 2 and 3, total RNA was extracted and subjected to quantitative real-time PCR. Immunohistochemical staining was carried our for confocal microscopy. Supernatants of differentiated clusters for used for radio immuno assays.
Results: Before induction of differentiation we found a strong expression of several stem cell markers including ABCG2 but also expression of Isl-1, a transcription factor known to be crucial for the development of pancreatic endocrine cells. Using immunocytochemistry, nuclear staining for ISL-1 was found in 10% of the expanded hMSC. Upon induction of differentiation, a step wise increase in the expression of Ipf-1 was found together with expression of insulin, amylin, glut-2 and somatostatin. In addition, a transitory expression of Ngn-3 and Pax-4 was observed as well as induction of Pax-6 along with glucagon. C-peptide was detectable immunohistochemically in the clusters. Additionally, in supernatants of differentiated islet like cluster we detected somatostatin.
Conclusion: Human bone marrow- derived MSC have the potential to generate islet- like clusters *in vitro* by recapitulating the coordinated, sequential expression of key genes observed during pancreatic development.

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In vitro trans-differentiation of rat bone marrow mesenchymal stem cells into islet-like cells
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Background and Aims: Effects toward routine islet cell transplantation have been hampered by islet availability as well as allograft rejection. Recent findings suggest that bone marrow mesenchymal stem cells (BM-MSC) have the capacity to differentiate into a variety of cell types including endocrine cells of the pancreas, which could provide an abundant source of autologous cells for this procedure. In this study, We investigate the trans-differentiation capacity of rat BM-MSC into islet-like cells.
Materials and Methods: Rat BM-MSC were isolated and cultured in DMEM supplemented with 10% FBS. CD45/CD90 expressions were detected by flow cytometry. In vitro differentiation of these BM-MSC under defined conditions resulted in spheroid cell clusters. Multiple genes related to islet-cells development and function(proinsulin, insulin, glucagon, somatostatin, pancreatic polypeptide, Glut-2, glucokinase, PDX-1, PAX-6, NKX2.2, GLP-1R) were detected by RT-PCR. Intra-cellular protein expressions of insulin, C-peptide, glucagon, somatostatin, pancreatic polypeptide were observed by laser con-focal microscopy and positive cell rates were deteced by flow cytometry. The ultrastructure of cell clusters were confirmed by electron microscopy.
Results: Following three to four passages, the BM-MSC became spheroid adherent monolayers with high CD90 positive rate(96.3 ± 1.3)% and very low CD45 expression(0.3 ± 0.4)%. After inducing, some spheroid cells formed islet-like clusters(d=80~200 μm) and half suspended in the culture medium. Electron microscopy revealed that secretory granules densely packed within the cytoplasm of the trans-differentiated cells. The spheroid cells expressed PDX-1, NKX2.2, PAX-6, GLP-1R, Glut-2, glucokinase, proinsulin, insulin, glucagon, somatostatin, pancreatic polypeptide gene and insulin, C-peptide, glucagon, somatostatin, pancreatic polypeptide proteins. The insulin-positive cells accounted for 10%~40%.
Conclusion: Rat BM-MSC could be trans-differentiated into islet-like cells in vitro and might represent a pool of cells for islet cell transplantation.

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Beta cell neogenesis I

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Induction of beta cell neogenesis by cytokines of the IL-6 family
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Background and aims: We recently reported a method to generate new beta cells from rat pancreatic exocrine tissue in a primary culture model. This was based on allowing exocrine acinar cells to transdifferentiate into metaplastic duct-like cells. These metaplastic cells are capable to transdifferentiate into beta cells in the presence of epidermal growth factor (EGF) and leukemia inhibitory factor (LIF). The beta cells obtained in this way are responsive to glucose and able to restore normoglycemia after transplantation in diabetic animals. For the present study we aimed at characterizing the signal transduction pathway involved in LIF-induced beta cell neogenesis.
Methods: Adult rat exocrine cells, depleted of contaminating beta cells, were cultured as monolayers in the presence of EGF and various LIF-related cytokines and/or specific inhibitors for 3 days. The cells were analysed at daily intervals by immunocytochemistry and RT-PCR for the expression of beta cell markers, transcription factors and signalling molecules.
Results: Ciliary neurotrophic factor (CNTF) was approximately equally potent as LIF in inducing neogenesis of insulin-positive cells, whereas interleukin-6 (IL-6) and IL-11 were ineffective. Up to 15% of the epithelial cells in the monolayers acquired beta cell characteristics (insulin, C-peptide-1, glut-2, Pdx-1) upon stimulation with both EGF and LIF or CNTF. Both cytokines require gp-130 and LIF-Rβ receptor activation for their action. Gp-130 and its downstream signal transduction molecules JAK2 and STAT3 and their phosphorylated (active) forms were expressed in the cell monolayers. Addition of the JAK2 inhibitor AG490 reduced beta cell neogenesis with approximately 90% whereas PpYLKTK-mts, a potent inhibitor of STAT3 phosphorylation, completely abrogated beta cell neogenesis. The neogenesis-inducing factors were found to induce expression of neurogenin-3 (Ngn-3), an important pro-endocrine transcription factor during embryonic development. Ngn-3 mRNA was absent in control cultures treated with only EGF and in PpYLKTK-mts-treated cultures. Hepatocyte nuclear factor-6 (HNF-6), an embryonic transcription factor known to activate Ngn-3, was also upregulated in the neogenesis-inducing conditions. Notch-1 receptor was strongly upregulated in the epithelial cultures, irrespective of whether EGF and/or cytokines were added or not. It could be that, as in embryonic development, Notch-signalling participates in the exocrine/endocrine fate decisions during metaplasia.
Conclusion: Beta cell neogenesis from adult metaplastic exocrine cells is induced by the combination of EGF and either LIF or CNTF. Intracellular signalling depends on activation of the gp130/LIFRβ-JAK2/STAT3 pathway and leads to expression of the pro-endocrine transcription factors HNF-6 and Ngn-3. It is not clear yet why at most 15% of the exocrine epithelial cells are re-programmed to the beta cell phenotype under these conditions, but the Notch-signalling system may be involved.

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The long-acting GLP-1 analogue, liraglutide, increases beta cell numbers during early human development
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Background and Aims: Glucagon-like peptide-1 (GLP-1) stimulates glucose-dependent insulin secretion, the primary reason that long-acting analogues have been developed for clinical use in type 2 diabetes. One such agent is liraglutide (Novo Nordisk A/S). Largely based on studies in rodents, GLP-1 has also been implicated in modulating adult beta cell mass by promoting beta cell neogenesis, proliferation and inhibiting apoptosis. To study these events in primary human cells, we have investigated GLP-1 signalling via liraglutide in the developing human fetal pancreas.
Materials and Methods: Human embryonic and fetal pancreas was collected at first trimester voluntary termination of pregnancy with ethical permission. Using this material, we have characterised potential GLP-1 signalling by RT-PCR. For culture experiments, tissue explants were prepared from the dorsal pancreas and cultured for 7 days in DMEM containing 10% fetal bovine serum in the presence or absence of 1 μM Liraglutide. After culturing, the explants were fixed, embedded and sectioned. Insulin-positive cells were counted in tissue sections from adjacent areas of the dorsal pancreas and expressed as a percentage of total epithelial cells per section. In repeat experiments, the pancreatic region from which explants were prepared was swapped between the liraglutide treatment and control groups.
Results: The pancreas was isolated at 8 weeks post-conception, when, from our earlier studies, it consists of epithelial pancreatic cells immediately prior to significant beta cell differentiation (Piper et al, *J Endocrinol*, 181, 11–23, 2004). By RT-PCR, the GLP-1 and GLP-2 receptors were expressed, whereas the glucagon receptor was less readily detected. Exposure to liraglutide increased the number of insulin-positive cells 1.95 +/– 0.17 fold (mean +/– S.E.; $p<0.001$). From early studies, it appears that this difference is not due to altered rates of apoptosis between treated and untreated specimens.
Conclusion: Future experiments will investigate proliferation within these samples, however the results from the present study imply that GLP-1 signalling, and more specifically liraglutide, increases primary beta cell number in the developing human pancreas.
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Monoclonal SP progenitors isolated from human fetal pancreas have phenotypic markers identical to mesenchymal stem cells
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Background and Aims: The side population (SP) phenotype might represent a common molecular feature for a wide variety of stem cells. Adult human islet-derived pancreatic progenitor cells contain SP cells. The aims of this study were to investigate whether monoclonal SP progenitor cells were established from human fetal pancreas and to detect their surface markers and their capability of proliferation and differentiation into pancreatic islet endocrine cells in vitro.
Materials and Methods: Islet-like cell clusters (ICCs) were isolated from human fetal pancreas. Monolayer epithelium-like cells were obtained from the ICCs and passaged thereafter. The SP cells or non-SP cells were sorted from these cells at the sixth passage after Hoechst 33342 staining. The culture of single SP cell or non-SP cell was assayed for clone formation. The surface markers and the capability of proliferation and differentiation of the cloned cells were further identified.
Results: The epithelium-like cells were able to be passaged for over 16 population doublings in vitro. The SP cells accounted for 0.1% of the total cells. RT-PCR revealed that ABCG2 mRNA was expressed in the sorted SP cells, but not in the non-SP cells. The rate of clone formation was about 2.7% for the SP cells, whereas there was no clone formation for the non-SP cells. The SP cell clones were further expanded for more than 15 passages and induced for differentiation into cells with characteristics of pancreatic β-cells. Furthermore, FACS analysis showed that CD44, CD90 and CD147 were positive, whereas CD34, CD38, CD45, CD71, CD117, CD133 and HLA-DR were negative on the monoclonal SP cells.
Conclusion: We show for the first time that the monoclonal SP progenitors are established from human pancreas and that the pancreatic SP progenitors share many phenotypic markers with mesenchymal stem cells derived from bone marrow. Therefore, this study may offer a novel method to purify pancreatic progenitor cells from human tissues and provide a good cell resource for stem cell research and for therapy of diabetes as well.
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Lentiviral PDX-1 mediated transdifferentiation of adult rat liver cells reverse plasma hyperglycemia in diabetic mice: ex-vivo cell therapy approach
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Background and Aims: *In vivo* expression of pancreatic duodenal homeoboxe1 (PDX-1) in mouse liver and *in vitro* expression into fetal human progenitor liver cells, were shown to turn them into insulin producing cells. However, implementing these techniques to humans has several draw-

backs. We aimed to construct a model of ex vivo gene therapy in rodents, based on *in vitro* transduction of primary mature hepatocytes with a human lentiviral vector carrying human PDX-1.
Materials and Methods: Primary rat hepatocytes were isolated by in vivo collagenase perfusion of the liver. The cells were transduced in primary culture with PDX1-lentiviral vector. Insulin expression and secretion of the newly engineered cells were assessed in vitro by RT-PCR, in situ hybridization, immunostaining and radioimmunoassay. PDX1-transduced hepatocytes were further studied *in vivo* by injecting them under the renal capsule of streptozotocin-induced diabetic SCID mice.
Results: Isolated rat hepatocytes were efficiently transduced in primary culture with the lentiviral vectors, as assessed by green fluorescent reporter gene (GFP) expression. The transduced cells exhibited insulin at both mRNA (RT-PCR, in situ hybridization) and protein levels (immunostaining and radioimmunoassay). Moreover the engineered cells presented a glucose and sulfonylurea dependent insulin secretion. Other β-cell genes expression was also detected: glucose transporter 2, glucokinase, sulphonylurea receptor 1, inwardly rectifying K^+ channel - KIR6.2 and prohormone convertase 1. When 3×10^6 cells were *in vivo* transplanted under the renal capsule of 7 streptozotocin-induced diabetic SCID-mice, significant reduction of non-fasting blood glucose levels from 552 ± 23 to 207 ± 38 (Mean±SEM, p=0.0002) was achieved in six weeks. No significant change in plasma glucose level was observed in control diabetic animals treated with lentivirus transfected cells only.
Conclusion: Lentiviral mediated PDX-1 expression in liver cells by ex vivo gene therapy is a potential mode for treating type 1 diabetes mellitus.
Support: Riva Foundation

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The time window of administration affects differently the development of the endocrine pancreas according the type of malnutrition
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Background and Aim: Human epidemiological studies have revealed the late consequences of malnutrition during gestation and early life on the health of the offspring. These studies have highlighted the inverse relationship between birth weight and the incidence of insulin resistance and type 2 diabetes later in life. Recent animal studies have demonstrated that protein restriction or calorie restriction disturb the fetal endocrine pancreas. Our aim was to examine if the time windows during which the diet is administrated would differently affect the development of the β cell mass in foetus.
Materials and Methods: Pregnant Wistar rats were fed either a low protein diet (8% of protein instead of 20%) during the entire gestation or during the last week of gestation or a low calorie diet (50% of the daily food intake) during the entire gestation or during the last week. Offspring were analysed after two weeks of gestation (F15) and on the last day of pregnancy (F21.5). Fetal β cell mass was measured by morphometrical analysis after immunohistochemistry for insulin. The expression of the differentiation markers (Ngn3, Pdx1, insulin and P48) was evaluated using immunohistochemistry
Results: Our data demonstrated that protein restriction as well as calorie restriction dramatically decreased the fetal beta cell mass. In the case of calorie restriction, the beta cell mass reduction was more pronounced when the restriction was given during the entire gestation ($p<0.01$) instead of during the last week ($p<0.05$). It was the opposite in the case of protein restriction. The beta cell mass was more reduced if the foetuses were protein restricted during the last week of gestation ($p<0.01$) compared to the entire gestation ($p<0.05$). Protein restriction also reduced the fetal beta cell proliferation rate on the last day of gestation ($p<0.05$). Regarding calorie restriction, the proliferation rate was not affected at that stage. At F15, the foetuses submitted to calorie restriction from the first day of gestation had a decrease in Ngn3 positive nuclei ($p<0.05$) and Pdx-1 ($p<0.01$, which was not observed in the protein restricted foetuses. At F21.5, in each group, all the Pdx1 positive cells in the islet express insulin, suggesting that the final maturation of the β cell was not affected by maternal malnutrition. At that stage, the labelling of exocrine pancreas by P48 was not affected in any of the groups, suggesting that there was no trouble of the commitment to the exocrine lineage.
Conclusion: We demonstrated that low calorie as well as low protein diet lower the fetal beta cell mass. However these two types of malnutrition act through different cellular and molecular mechanisms. These differences may explain the variable impact of the time windows in the two models. The low protein diet didn't affect the differentiation of the pancreatic lineage but disturbed the fetal β cell proliferation as observed in late gestation. Consequently, the effect of the low protein diet did not appear more marked if the time window is extended to entire gestation, compared to the last week. On the opposite, the low calorie diet did not affect the proliferation but modified the expression of markers of the pancreatic lineage. This could explain that the influence of extending time windows is higher in this case.

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Notch expression during pancreatic exocrine cell metaplasia preceding beta-cell neogenesis
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Background and aims: Notch determines the preservation and the cell fate of common exocrine and endocrine precursor cells in fetal pancreas. The present study investigates the expression and role of Notch in exocrine cell metaplasia that precedes beta-cell regeneration in adult rat pancreas.
Materials and methods: We studied acino-ductal cell metaplasia in established beta-cell regeneration models in rat pancreas: In vivo after ligation of exocrine ducts, and in a culture system where beta-cells can be generated from metaplastic exocrine cells. RNA and protein analyses were performed.
Results: During the initiation of beta-cell regeneration after duct-ligation and in primary culture, fully differentiated acinar exocrine cells become metaplastic: they lose their characteristics like expression, storage and secretion of zymogens, and gain a new profile of transcription factor expression. The changes in transcription factors include a reduction of p48/Ptf1a, loss of Mist1 and gain of Pdx1. Sel-1L, a negative regulator of Notch expression is down-regulated. Concomitantly, Notch1 receptor, its ligands Dll1, Jagged1 and Jagged2, and the target genes Hes1, Hey1 and Hey2 become highly expressed.
Conclusions: Our data show that Notch-signalling is activated in metaplastic exocrine cells during conditions of induced pancreas and islet beta-cell regeneration. Concurrently, expression of Sel-1L, an inhibitor of Notch activity, is suppressed. We previously showed that adult exocrine cells can act as precursors of islet beta-cells during metaplastic conditions. We conclude that the Notch embryonic cell specification system can also function in adult pancreas to regulate regeneration.

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Splenocytes enhances islet regeneration in diabetic rats with a high fat diet
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Background and Aims: Recent studies showed that possible neogenic sources of β-cell may be ductal cells, splenocytes or bone marrow cells in rodent diabetic models. We determined whether splenocytes played an important role in insulin resistance and β-cell mass and function in type 2 diabetic rats administered with 15 En% sugar (SU), 40 En% fat (HF) and control (20% fat) diets for 5 weeks.
Materials and Methods: Female 90% pancreatectomized Sprague Dawley rats were randomly divided into three groups: 1) removal of spleen (NSP), 2) removal of spleen + injection of male splenocytes (ISP), and 3) sham operation (Sham). Each group was sub-divided into three groups, and the respective groups were treated with three different diets for 5 weeks. At the end of experimental period, insulin secretion capacity was measured by hyperglycemic clamp. At 6 hours after $BrdU^+$ injection, pancreas was fixed with 4% paraformaldehyde to perform immunohistochemistry.
Results: Compared to control diets, HF and SU diets increased the first and second phase insulin secretion in Sham rats by 31.8 ± 5.2 and 42.5 ± 4.9%, respectively, and in ISP rats by 48.5 ± 5.3 and 52.4 ± 6.4, respectively. However, HF diet decreased the secretion only in the NSP group. The changes of β-cell mass revealed as the same pattern of the insulin secretion. HF significantly increased the apoptosis of β-cells and decreased the proliferation regardless of spleen removal, but not SU and control groups. Fish analysis revealed nuclei positive for the Y chromosome within islets and ductal cells in rats in the ISP group. The β-cell proliferation determined by $BrdU^+$ incorporation was not affected by spleen removal in all three diets. The number of single cells and small β-cell clusters indicating neogenesis of β-cells significantly increased in ISP and Sham with HF diets, but it was not altered in NSP with HF diet. Thus, decreased β-mass in the NSP-HF diet was due to decreased neogenesis of β-cells in spite of increased apoptosis. The β-cell mass and neogenesis of the ISP-HF was increased up to those of the Sham-HF group.
Conclusion: Sufficient β-cell mass is required to increase insulin secretion capacity in insulin resistant states. Splenocytes are an important source for neogenesis β-cells in 90% pancreatectomized rats, especially in high-fat induced insulin resistant states.

PS 20
Beta cell neogenesis II

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Neogenesis, distribution and ageing of pancreatic β-cells in insulin resistant and diabetic *Macaca mulatta* monkeys

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Background and Aims: Changes in β-cell turnover resulting from an inbalance of neogenesis and apoptosis are considered as aetiological factors for Type 2 diabetes and have been demonstrated in rodents: β-cells associated with ductal epithelium (sites of stem cells) have been demonstrated in adult as well as fetal pancreas and increased β-cell apoptosis is found in diabetic rodents. *Macaca mulatta* monkeys spontaneously develop obesity and diabetes and are a good model for examination of β-cell neogenesis and turnover in relation to increased insulin resistance and diabetes. In addition, ageing of primate β-cells can be assessed from accumulation of secondary lysosomes as has been shown as a marker for ageing of neurones. The aims of this study were to determine how β-cell neogenesis, distribution and ageing were related to the pathophysiology of the diabetic syndrome in this primate model.
Materials and Methods: Pancreatic specimens were obtained at necropsy at different stages of the diabetic syndrome from animals bred and housed in captivity. Tissue was prepared for immunocytochemistry (light microscopy) and electron microscopy (EM). Monkeys were classified as normal (Grp1, n=7), insulin resistant (Grp2, n=8) or diabetic (Grp3, n=9) from fasting glucose and insulin concentrations. Tissue sections were labelled for insulin and with a marker for ductal cell (anti-CK19). Quantification included distribution of β-cells within islets, clusters and ducts. Secondary lysosomal area (size) and lysosomal area as a proportion of β-cell cytoplasm was determined from EM images of islets from normoglycaemic monkeys of different ages (range 5–30 yrs) at necropsy.
Results: Islet density/pancreas was unchanged between groups (Grp1, Grp2, Grp3) and islet area proportion was increased in Grp2 (mean 4.52%) compared to Grp1 (2.36%;$p<0.05$). Islet β-cell proportion was increased in Grp2 and reduced in Grp3 compared to Grp1 ($p<0.05$). The density of ductal β-cells was lower in diabetic (Grp3) than in normoglycaemic animals (Grps 1,2). β-cell proportion /islet was increased in Grp2 compared to Grp1 ($p<0.05$). There was a positive relationship between animal age and the number of secondary lysosomes ($r=0.891$) and their area proportion ($p<0.01$).
Conclusion: In the primate diabetic syndrome, islet size increases with increased insulin resisitance by β-cell hyperplasia with no overall changes in islet number. Islet β-cell proportion is reduced in amyloid-containing islets in diabetes but ductal β-cell neogenesis is not increased. An increase in lysosomal number and size as markers of cellular age indicate increasing longevity of β-cells suggesting that low levels of cellular turnover occurs in primates.

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Gastrin-induced insulin production in hepatocyteG2 cells

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Background and Aims: Transplantation therapy of diabetes mellitus is hampered by the lack of pancreatic donor tissue. Liver cells amended to produce insulin may be regarded as an alternative tissue source for transplantation. Using a human hepatoma cell line as a model system, we tried to initiate insulin production in these cells by methods not requiring gene transfer technology.
Materials and Methods: HepG2 cells (from ATCC) which express multiple markers characteristic for hepatic precursor cells were cultured either as rapidly proliferating plastic-adherent cells in D-MEM/10% FCS, or as free-floating multicell „spheroids" about 100–250 µm in diameter. Insulin gene expression was monitored by immunohistology, RT-PCR, and ELISA for human C-peptide. The C-peptide ELISA (from Mercodia, Uppsala) was proven not to react with any of the media or chemicals used in this study.
Results: HepG2 cells propagated as adherent cultures showed strong nestin expression in both RT-PCR and immunocytology. Spontaneous, but rather weak insulin gene expression was demonstrated by RT-PCR and C-peptide ELISA (<1 ng/mg cell homogenate protein). Cellular hormone content could slightly be enhanced by high glucose, but not by additional supplementation of the culture medium with gastrin or other drugs. Mechanically prepared HepG2 multicell aggregates formed free-floating „islet-like" spheroids when cultured in serum-free medium. In these spheroids, cells stopped proliferation and remained viable for at least 14 days when cultured in high glucose (4.5 mg/ml) serum-free Neurobasal A/B27 medium (Gibco). Addition of gastrin and EGF or gastrin and GLP-1 to the culture medium enhanced cellular C-peptide levels up to 9.2 ± 1.5 ng/mg protein, n=6, or 16.2 ± 3.0 ng/mg protein, n=6, respectively. In contrast, many other substances (such as nicotinamide, retinoic acid, IGF-1, NGF, VEGF, db-cAMP, dexamethasone, glucagon, Ly 294002, gamma-secretase inhibitor) which were supposed to support beta-cell development in other systems did not significantly enhance cellular C-peptide levels over those found in control cultures (2.4 ± 0.4 ng/mg protein, n=12). Hepatocyte growth factor overcame the cell proliferation block and thus led to much larger, but ischemically damaged spheroids. C-Peptide release from spheroids cultured in gastrin/EGF supplemented Neurobasal A/B27 medium, and then preincubated for 4 hours in low-glucose TCM-199, could be stimulated by glucose plus cAMP-enhancing drugs, but not by glucose alone. In immunocytology, a fraction of the spheroid cells stained positive for insulin C-peptide, and most of the cells showed strong reaction for PGP9.5, a neuroendocrine marker strongly expressed also in adult human endocrine islet cells.
Conclusion: Free-floating multicell spheroids formed from HepG2 cells may serve as an easily accessible model system to study methods for inducing insulin gene expression in hepatic cells.

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Regeneration of pancreatic β-cells in L-type calcium channel α_{1D} subunit ($Ca_v1.3$) heterozygous knock out mice after 90% partial pancreatectomy

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In the recent report, homozygous knock out mice of L-type calcium channel α_{1D} subunit ($Ca_v1.3$), which plays a crucial role in insulin secretion, showed impairment of postnatal pancreatic β-cell development as well as insulin secretion. We performed 90% partial pancreatectomy(Px) in heterozygous $Ca_v1.3$ knock out mice to investigate the effect of partial deficiency of $Ca_v1.3$ gene on β-cell regeneration in the adult. Glucose homeostasis including intraperitoneal glucose tolerance test and insulin tolerance test, metabolic profiles including serum insulin and lipid levels and morphologic changes of pancreatic islets were studied. There was no difference of the volume of pancreatic regeneration at 3 days and 8 weeks after 90% partial Px. 90% Partial Px induced glucose intolerance only in the heterozygous knock out mice at 8 weeks after surgery. Distribution of islet size in remnant pancreas was significantly different between two groups at 8 weeks after partial Px; median value of islet size of heterozygote was larger than that of wild type (642.8 μm^2 vs 1459.8 μm^2, $P<0.01$). The frequency of single β-cell unit, considered as a marker of β-cell neogenesis, was much lower in heterozygote than that of wild type (41% vs 23.3%, $P<0.05$). These data suggest that $Ca_v1.3$ gene deficiency is specifically associated with impairment of beta cell neogensis and eventual glucose intolerance in the 90% partial pancreatectomized mice.

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Growth of beta cell mass in implants of endocrine cells purified from prenatal porcine pancreas

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Background and Aims: Shortage in human donor pancreases has raised interests in porcine organs as potential source of islet cell grafts for use in patients. Porcine islet tissue has been isolated from donors of different ages and shown to correct diabetes in immune deficient mice. The islet cell clusters from fetal and neonatal organs are low in endocrine cell content but form implants that can grow in beta cell mass and/or insulin content. It is unclear whether, and if so, to which extent, this growth results from neoge-

nesis out of non-endocrine cells or from proliferation of existing beta cells. This study investigates whether beta cell growth occurs in implants of endocrine cells purified from prenatal porcine organs, and compares the relative contributions of beta cell proliferation and of an increase in individual beta cell volume.
Materials and Methods: Porcine endocrine islet cells were purified from fetuses at gestation day 110–115. Grafts consisting of 0.5 to 1 million beta cells were implanted under the kidney capsule of normal nude mice. The number and cellular volume of the beta cells was monitored over 20 weeks. Beta cell functions were analyzed by following their proliferation activity through BrdU incorporation, their secretory activity through plasma porcine C-peptide levels, their cellular insulin storage through immunoassay of the implant.
Results: At the end of posttransplantation (PT) week 1, the total volume of the beta cell population was similar to that on PT day 1; this was also the case for the number of beta cells and for the average volume of individual beta cells. However, the rate of beta cell proliferation was high during this first week -reaching peak values of 5 ± 0.7% BrdU-positive cells at PT day 3. The rate of apoptosis was also elevated. These data suggest that beta cell losses during engraftment are compensated by formation of new beta cells through proliferation of existing beta cells.
Between the end of PT week 1 and PT weeks 10 and 20, total beta cell volume in the implants increased, respectively, 3- and 9-fold. This overall rise was the result of a 4-fold increase in the number of beta cells and a nearly 2-fold increase in their individual cell volume. Over this period, the rates of beta cell proliferation in the implants remained 4 to 12-fold higher than in pancreatic tissue, for low rates of apoptosis; individual beta cells progressively increased their insulin content up to 6-fold the levels at start.
From PT week 5 on, porcine C-peptide was detected in plasma, rising to plateau levels of 1 to 2 ng/ml. At this stage of development, the implants were capable of correcting diabetes.
Conclusion: Perinatal beta cells exhibit a potent growth potential both in terms of proliferation as of increase in individual cellular volume and insulin content. When used in grafts, this potential can first compensate for beta cell losses during engraftment and then develop a beta cell mass that, upon functional maturation, becomes capable of correcting diabetes. Our observations are consistent with recent work in mice showing that beta-cells are the major source for postnatal growth of the beta cell mass. The presently used beta cell preparations provide a model for investigating the regulation of beta cell growth and maturation.
Support: The Juvenile Diabetes Research Foundation, the Belgian Fund for Scientific Research, the Institute for the Promotion of Innovation by Science and Technology in Flanders, the Interuniversity Attraction Poles from the Belgian Science Policy

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Proteomic analysis of differential protein expression in response to epidermal growth factor in neonatal porcine pancreatic cell monolayers

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We have proposed that porcine neonatal pancreatic cell clusters (NPCCs) may be a useful alternative source of cells for islet transplantation, and that monolayer cultures might provide an opportunity to manipulate the cells before transplantation. In addition we previously identified 10 genes up-regulated by epidermal growth factor (EGF) in cultured porcine NPCC monolayers. We have now analyzed the intracellular signaling pathways activated by EGF and searched for proteins differentially expressed following EGF treatment of the monolayers, using two-dimensional gel electrophoresis and matrix-assisted laser desorption/ionization-time of flight mass spectrometry. EGF treatment resulted in phosphorylation of both Erk 1/2 and Akt, as well as increased cell proliferation. Five unknown and 13 previously identified proteins were differentially expressed in response to EGF. EGF treatment increased the expression of several structural proteins of epithelial cells, such as cytokeratin 19 and plakoglobin, whereas vimentin, the intermediate filament protein of mesenchymal cells, and non-muscle myosin alkali chain isoform 1, decreased. Heterogeneous nuclear ribonucleoprotein (hnRNP) A2/B1 factor, which promotes epithelial cell proliferation, and hemoglobin alpha I & II also increased, whereas cyclin A1, immunoglobulin heavy chain, apolipoprotein A1, 5,10-ethylenetetrahydrofolated reductase (5,10-MTHFR), angiotensin-converting enzyme 2 (ACE2), co-lipase II precursor, and NAD^+ isocitrate dehydrogenase (NAD^+ IDH) alpha chain proteins decreased. Our results show that EGF stimulates proliferation of pancreatic epithelial cells by simultaneously activating the MAPK and PI-3K pathways. HnRNP A2/B1, hemoglobin, cyclin A1 and ACE2 may play roles in the proliferation of epithelial cells in response to EGF.

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Adenoviral overexpression of insulin-like growth factor II modulates β-cell replication and β-cell apoptosis in rat pancreatic islet exposed to interleukin-1β

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Background and Aims: Interleukin-1β (IL-1β) is a pro-inflammatory cytokine that has been shown to inhibit islet β-cell function, to induce β-cell apoptosis and to suppress β-cell replication. Insulin-like growth factor-II (IGF-II) is a growth promoting peptide which is able to stimulate β-cell proliferation, differentiation and survival. The aim of this study was to determine the effect of IGF-II overexpression on β-cell replication and β-cell apoptosis in adult pancreatic islets exposed to different concentrations of IL-1β.
Materials and Methods: Islets from Lewis rats (6–8 w.o) were infected *ex vivo* with Ad-IGF-II, incubated overnight in serum-containing medium at 11.1 mM glucose, and cultured for 48 h in RPMI-1640, 10% FCS at 5.5 or 22.2 mM glucose with or without 10, 30 and 50 U/ml of IL-1β.
Results: β-cell apoptosis, measured by the TUNEL technique, was increased after 48 h exposure at all IL-1β concentrations in a dose-dependent manner. In islets cultured at 5.5 mM glucose, exposure to 10 U/ml IL-1β was sufficient to increase β-cell apoptosis (control: 0.30 ± 0.04%, 10 U/ml IL-1β: 0.76 ± 0.07%; $p<0.005$). In contrast, in islets incubated at 22.2 mM glucose 30 U/ml of the cytokine were needed to induce β-cell apoptosis (0.88 ± 0.1%; $p<0.03$). Overexpression of IGF-II protected the islets from IL-1β-induced apoptosis, and higher concentrations of the citokine were needed to increase β-cell apoptosis: 30U/ml (0.82 ± 0.2%, $p<0.05$) in islets cultured at 5.5 mM glucose and 50U/ml (0.99 ± 0.1%, $p<0.01$) in islets cultured at 22.2 mM glucose. β-cell replication was significantly reduced when islets were exposed to IL-1β, and a similar reduction was found at both glucose concentrations. When islets were exposed to 10 U/ml IL-1β, inhibition of β-cell replication was already found, (5.5 mM glucose: control: 0.74 ± 0.1%; 10 U/ml IL-1β: 0.15 ± 0.05%, $p<0.005$), (22.2 mM glucose: control: 2.07 ± 0.1%; 10 U/ml IL-1β: 0.56 ± 0.2%; $p<0.005$) and replication was almost abolished in islets exposed to 30 U/ml IL-1β. β-cell replication was significantly higher in Ad-IGF-II infected islets cultured at both glucose concentrations, but IGF-II overexpression reduced the negative effect of the cytokine on β-cell proliferation only in islets exposed to 10 U/ml IL-1β (5.5 mM glucose: 0.5 ± 0.2%, 22.2 mM glucose: 1.62 ± 0.5%), but not in islets exposed to 30 and 50 U/ml of the cytokine.
Conclusions: The deleterious effects of IL-1β on β-cell replication and apoptosis occurred in a dose-dependent manner. β-cell replication was more sensitive than β-cell apoptosis to the deleterious effects of IL-1β. IGF-II overexpression resulted in a partially protection against IL-1β-induced β-cell apoptosis at all IL-1β concentrations used, but was able to preserve β-cell replication only in islets exposed to 10 U/ml IL-1β. Considering that the effects of IL-1β may be relevant in the development of type I diabetes and could contribute to the β-cell loss that takes place after islet transplantation, IGF-II may be useful in the design of strategies to preserve β-cell mass in these conditions.
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Gluco-conditioned MIN6 pseudoislets: a physiological in vitro model of primary pancreatic islets

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Background and Aims: The limited availability of primary human islet tissue for research currently inhibits progress in developing transplantation as a successful therapy in diabetes. In vitro studies using insulin-secreting cell lines are of limited value to the understanding and modelling of transplantation and diabetes since such cell lines are phenotypically very different from primary pancreatic islets. Cells are routinely studied as monolayers in media containing supra physiological concentrations of glucose, typically 10–25 mM. We have previously carried out work using clusters of MIN6 cells (pseudoislets) as a model for primary islets. The pseudoislets (PIs) resemble primary islets in both size and function and are able to

maintain both their islet-like morphology and responses to external stimuli over extended culture (28+ days). Previously, both cell-lines and PIs required routine maintainance in 25 mM glucose-containing media. The limitations of studying the effects of diabetogenic glucose exposure in cells that have been chronically exposed to high glucose are obvious.
Materials and Methods: In this study, we exposed PIs to selective passage and serum withdrawal to create a PI that was conditioned to physiological levels of glucose. MIN6 cells were deprived of FCS for 3 days to synchronise cell cycle and passaged over 4 weeks in decreasing glucose concentrations (22–5.5 mM glucose). Cells were then frozen at –70 °C for three weeks and when defrosted were placed in media containing 10%FCS and 5.5 mM glucose. MIN6 cells and PIs were then cultured for 7 days in the presence of 5.5, 11 or 22 mM glucose-containing media. Viability, apoptosis and necrosis were determined using a combination of Hoescht/Propidium Iodide dyes.
Results: PI viability was similar at 5.5 mM and 11 mM glucose levels but was significantly reduced at 22 mM glucose ($73 \pm 4\%$ v $62 \pm 3\%$, $p \leq 0.05$). Apoptosis and necrosis levels remained unchanged. MIN6 monolayers and PIs showed similar properties at 5.5 mM glucose. At 11 mM glucose, viability remained the same but monolayer apoptosis was significantly lower compared to PIs ($9 \pm 1\%$ v 17 ± 2, $p \leq 0.05$). At 22 mM glucose, monolayers had greater viability than PIs ($78 \pm 3\%$ v 62 ± 3, $p \leq 0.01$) and lower apoptosis ($17 \pm 2\%$ v 28 ± 3, $p \leq 0.05$). Necrosis levels were not significantly different at 5.5 mM, 11 mM or 22 mM glucose.
Conclusion: Gluco-conditioning has enabled successful culture of MIN6 cells in physiological levels of glucose with further enhancement of viability and function in PI configuration, thus establishing a superior *in vitro* model of primary pancreatic islets.

PS 21
Transcriptional regulation in beta cells

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The liver receptor homolog 1 is expressed in islets and its transcription is regulated by PDX-1
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Background and Aims: Liver receptor homolog 1 (LRH-1) and pancreatic-duodenal homeobox 1 (PDX-1) are coexpressed during pancreatic development when PDX-1 regulates LRH-1 expression. In adult rodents, LRH-1 was considered to be expressed only in the exocrine pancreas. The present study aims at identifying LRH-1 in the endocrine pancreas and its putative effects on insulin secretion and beta-cell gene expression in INS-1 cells and rat pancreatic islets.
Materials and Methods: We have employed the Tet-On system to establish two INS-1 stable cell lines permitting, respectively, overexpression or dominant-negative (DN) suppression of LRH-1 function. The DNLRH-1 retains DNA-binding but lacks the transactivation capacity. Two stable INS-1 clones called LRH-1 #17 and DNLRH-1 #25 were selected for the present study. We have also generated a recombinant adenovirus allowing inducible expression of human LRH-1. Collagenase-isolated rat islets were infected for 48 h in the presence or absence of the inducing agent doxycycline. Northern blotting was performed on INS-1 cell lines, including those allowing overexpression or suppression of PDX-1 function.
Results: RT-PCR analysis showed LRH-1 expression in rat islets and FACS-sorted beta-cells at levels 10 times lower than in rat liver. Immunofluorescence staining and immunoblot analysis with an anti-LRH-1 antibody revealed the presence of endogenous LRH-1 in INS-1 cells, which was less abundant in islets. Both wild-type and DNLRH-1 proteins were induced dose-dependently by doxycycline. Overexpression of PDX-1, HNF-1alpha or HNF-4alpha in stable INS-1 clones caused the expression of different transcripts of LRH-1. Conversely, upregulation or suppression of LRH-1 function did not alter the expression of insulin or genes regulating glucose metabolism, insulin exocytosis or lipogenesis. Glucose-stimulated insulin secretion in INS-1 cells was attenuated only after robust induction of LRH-1, whereas insulin release in islets was unaffected. In contrast to reports on hepatoma cells, LRH-1 did not stimulate DNA synthesis in INS-1 cells or islets, assessed by BrdU incorporation.
Conclusion: The transcription factor LRH-1 is expressed in beta-cells and the derived INS-1 cells. The factor does not affect the expression of key beta-cell genes or control beta-cell proliferation. Alteration of PDX-1 expression determines LRH-1 transcript species, the downstream effects of which require further investigation.

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Glucose-induced interaction partners and a new kinase of PDX-1
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Background and Aims: PDX-1 is a transcription factor mediating glucose-signalling to the insulin gene promoter. A novel class of NLS within the third helix of the homeodomain of PDX-1 characterised in our lab mediates its nuclear localisation. Nuclear transport of PDX-1 is induced by stimulating serum- and glucose-starved beta cells with a high dose of glucose. The aim of our study was to identify glucose-induced interaction partners of PDX-1.
Material and Methods: Pulldown experiments were performed using bacterially expressed GST-PDX-1 protein as a bait to precipitate interacting cytoplasmic proteins from glucose stimulated ^{32}P-labelled MIN6 cells. Captured proteins were subjected to 2D-gel electrophoresis and analysed by MALDI-TOF. To map phosphorylation sites, *in vitro* kinase assays with deletion and point mutants of PDX-1 as substrates were performed and the localisation of PDX-1 was determined by immunofluorescence experiments.
Results: 14-3-3ε was identified as a PDX-1 interacting protein. The interaction has been verified in GST-capture assays as well as immunoprecipitation experiments and domains involved have been elucidated.
Furthermore, we show that Casein Kinase 2 (CK2) is able to phosphorylate the PDX-1 protein. Both recombinant and endogenous CK2, immune pre-

cipitated from glucose-induced MIN6 cells, phosphorylate GST-PDX-1 in an *in vitro* kinase assay. This phosphorylation could be diminished by addition of Emodin, a specific inhibitor of CK2. The IC_{50} values for this inhibition range from 2 μM for recombinant CK2 to 20 μM for the immune precipitated kinase. Time course experiments showed that the PDX-1 phosphorylation by CK2 peaks 5–10 min after glucose stimulation of MIN6 cells.
In contrast to MAPK, CK2 phosphorylates strongly the homeodomain of PDX-1. Point mutants of the PDX-1 homeodomain revealed S173 as a phosphorylation site for CK2. Mutation of the serine at the position 173 to alanine abolishes the phosphorylation of the PDX-1 homeodomain.
Conclusion: In the present study we demonstrate that CK2 and 14-3-3ε are able to interact specifically with PDX-1 *in vitro* and that the phosphorylation of PDX-1 by CK2 allows its binding to the A-box of the insulin gene promoter. Both interaction partners are affected by glucose concentration and may therefore integrate glucose derived signals to PDX-1 at different levels. These results suggest an involvement of CK2 and 14-3-3ε in the signalling pathway leading to the activation of the insulin gene expression via PDX-1.

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Ameliorating effect of fenofibrate on INS1 cells impaired by palmitic acid associated with up-regulated PDX-1 expression
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Background and Aims: Fenofibrate (FF), an agonist of peroxisome proliferator-activated receptor alpha (PPARα), is a representative drug used in hyperlipidemias and diabetes mellitus therapy. Some studies support the idea that PPARα activity was one way of lipitoxicity. However, others testified the function deficiency in beta cell induced by fatty acids was improved by treated with PPAR α agonist. Pancreas-duodenum homeobox-1 (PDX-1) transactivates insulin and plays an important role in maintaining beta cells normal function. In addition, it has been shown free fatty acids impair insulin secretion by inhibiting PDX-1. The present study aimed to explore the effects of FF on PDX-1 in INS1 cell line suffered toxicity of palmitic acid (PA).
Materials and Methods: INS1 cells were divided into 4 groups: control group, 0.2 mM PA group(PA), 5×10^{-6}M FF group(FF), 0.2 mM PA with 5×10^{-6}M FF treatment group(PF). After cultured for 24 hours, INS1 cells were incubated in Krebs Ringer bicarbonate buffer containing 3 mM or 20 mM glucose for 20 minutes, aliquots of the incubation buffer were retrieved for assay of insulin concentration by RIA and then calculated BIS and GSIS/BIS ratio. The mRNA levels of pancreas-duodenum homeobox-1(PDX-1) and glucose transport protein 2(GLUT-2) and PPARα were determined by real-time PCR. GLUT-2 and PDX-1 protein levels were measured by western blot. GLUT2 protein was further detected by immuno-fluorescence assay. The PDX-1 bonding activity with insulin I promoter A1 box was detected by electrophoretic mobility shift assay (EMSA).
Results: 1. PA increased BIS 31% and impaired GSIS/BIS ratio (2.56 fold vs. 3.3 fold) as compared with control group ($P<0.05$); FF had no significant effect on BIS and GSIS/BIS ratio ($P>0.05$). In PF group, BIS was decreased significantly and GSIS/BIS ratio (3.2 fold) was improved as compared with PA group ($P<0.05$). 2. GLUT2 mRNA was down-regulated in PA group as compared with control group ($P<0.05$),but restored in PF group. Opposite the mRNA changes, there were no strong difference in all groups in GLUT2 protein expression either by western blot or by immuno-fluorescence assay ($P>0.05$). 3. PPARα mRNA levels of FF group and PF group were enhanced. 4. FF had no effect on both PDX-1 mRNA and protein level but increased binding activity slightly. PDX-1 mRNA, protein level and its binding activity with insulin promoter A1 box were deeply reduced in PA group but were markedly improved in PF group (all $P<0.05$).
Conclusion: Fenofibrate improved INS1 cell secretion function defect-caused by palmitic acid. This effect is correlated with enhancements of PPAR α or PDX1 mRNA, protein concentration and activity.
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Activation of the rat insulin gene II in insulinoma cells is repressed by Pax6 which competes with PDX-1 and interacts with BETA2
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Background and Aims: The transcription factor Pax6 was shown to be important for the maintenance of pancreatic beta-cell function. It acts as a transactivator of rat insulin I gene by binding to the promoter PISCES element. But this sequence element is not present in the rat insulin gene II promoter or in mouse and human insulin gene promoters.
Materials and Methods: We used the yeast two hybrid system and GST capture assays to identify proteins interacting with Pax6. Gel shifts and chromatin immunoprecipitations were done to specify Pax6 binding sites within the rat insulin gene II promoter. The Dual Luciferase Assay system and insulin radioimmunoassay were used to prove the importance of this transcription factor for insulin gene expression and secretion. Regulation of the intracellular localisation was visualised applying fluorescence microscopy.
Results: We found that Pax6 interacts with BETA2 via its paired box. Furthermore, we show that Pax6 competes with PDX-1 for binding to the promoter A-elements. Over expression of Pax6 prevents the synergistic activation of the rat insulin II gene expression triggered by BETA2 and PDX-1 and results in a decreased insulin secretion. Moreover, we observed a predominantly nuclear localisation of Pax6 in INS-1 cells under low glucose concentrations, whereas under high glucose concentrations the protein is predominantly localised in the cytosol. The glucose dependent localisation of Pax6 is reversely to that of PDX-1.
Conclusion: Low concentrations of glucose lead to increased concentrations of Pax6 in the nucleus disabling the activation of insulin gene expression by interacting with BETA2 and competing with PDX-1 at the promoter A-box.

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Molecular mechanisms underlying the effect of *all-trans* retinoic acid on glucokinase gene transcription in insulin-producing cells
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Background and Aims: Retinoic acid (RA) has a vital role in islet development and beta-cell function. RA increases insulin production and secretion both in islets and beta-cell lines. Repletion of vitamin A-deficient rats with RA improves their insulin response to a glucose challenge. It has been shown that RA increases both glucokinase activity and mRNA-levels in beta-cells, however the molecular mechanism behind these effects remained unclear. The aim of this study was to elucidate the molecular basis of the stimulatory effect of RA on beta-cell glucokinase (bGK) gene transcription at the level of the involved *cis*- and *trans*-elements.
Materials and Methods: To investigate the effect of *all-trans* RA on bGK expression we employed comparative RT-PCR, on-line monitoring of bGK promoter-driven GFP fluorescence and electrophoretic mobility shift assays (EMSA).
Results: Stimulation of rat islets or beta-cell lines with 1 μM RA leads to a 1.5- to 3-fold increase in bGK mRNA and bGK promoter-driven GFP expression. Mutation of the TGGT-motifs (TGGT1 at -90 bp, TGGT2 at -165bp) in the bGK promoter leads to a reduction in basic bGK expression and to complete loss of the response to RA. RA-stimulated bGK promoter activity was increased 2-fold when RARalpha and RXRalpha were overexpressed in beta-cells. RA treatment enhances DNA-binding of beta-cell nuclear proteins to the TGGT-motifs in EMSA assays. Antibodies against RAR were able to super-shift these retarted DNA-protein complexes.
Conclusion: We now demonstrate that RA stimulates bGK gene transcription via a mechanism involving binding of the retinoic acid receptor RAR to the two TGGT-motifs of the bGK promoter.

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Spatial segregation of insulin receptor B-type signaling allows selective and simultaneous activation of glucokinase and c-fos gene transcription
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Background and Aims: Insulin exhibits pleiotropic effects that involve mitogenic and/or metabolic events. To achieve selectivity in insulin action

at the single cell level, the temporal and spatial resolution of signaling events is required. In the pancreatic β-cell, we have recently shown that selective insulin signaling via the 'metabolic branch' involves the insulin receptor (IR)-A isoform to up-regulate the transcription of the insulin gene, while the IR-B isoform is involved in activation of the β-cell glucokinase (βGK) gene. One mechanistic basis to achieve selectivity in signal transduction via IR-A and IR-B is the localization of the two receptor isoforms in different plasma membrane domains and the subsequent access to different adapter proteins. While this may explain different signaling via IR-A and IR-B, it remains to be explored how selectivity is achieved when utilizing the same IR isoform.
Gene expression profile analysis of insulin producing cells in response to insulin stimulation revealed, among other genes, the up-regulation of the proto-oncogene c-fos, which is activated via the 'mitogenic branch' of insulin signaling.
The first aim of the present study was to analyze which IR isoform contributes to the activation of c-fos gene transcription by insulin in the pancreatic β-cell. The second aim was to address the more general question how selectivity in signal transduction can be achieved when signal transduction originates from the same receptor isoform.
Materials and Methods: We employed promoter-driven GFP and DsRed expression as a read-out system in combination with application of selective antibodies or pharmacological inhibitors as well as transient co-expression studies. To clarify the signaling cascade involved, co-expression of epitope tagged wild type and mutant variants of IR-A and IR-B, as well as wild-type and dominant-negative variants of potential downstream adapter proteins (p85 and p52-Shc) were applied. Furthermore, to investigate the requirement of IR-endocytosis in insulin-stimulated c-fos gene transcription, we co-expressed dominant-interfering dynamin2, epitope tagged wild type and mutant early and late endosome markers Rab5 and Rab7 and the μ2-subunit of AP2. Insulin promoter-driven DsRed expression and βGK promoter-driven GFP expression served as internal controls for signal transduction via IR-A and IR-B, respectively.
Results: Insulin-stimulated transcription of c-fos and βGK genes is activated simultaneously in the pancreatic β-cell via IR-B. While βGK gene transcription requires the integrity of the IR-B juxtamembrane NPEY-motif and signaling via a PI3K C2α-like activity/PDK-1 and PKB, c-fos promoter activation is dependent on the intact carboxyterminal YTHM-motif of IR-B and signaling via PI3K Ia/p52-Shc/MEK1 and ERK1/2. Moreover and most interestingly, analysis of both promoters revealed that insulin activates βGK gene transcription from membrane-standing IR-B, while c-fos promoter activation is dependent on IR-B-endocytosis.
Conclusion: By comparing the mechanisms that allow simultaneous activation of c-fos and βGK genes, we provide evidence that selectivity in insulin signaling via the same IR isoform can be achieved by signal transduction arising from different cellular compartments.

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Carbohydrate response-element binding protein regulates liver-type pyruvate kinase and pancreatic duodenum homeobox-1 expression in MIN6 β-cells by direct and indirect mechanisms, respectively
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Background and Aims: Carbohydrate Response-Element Binding-Protein (ChREBP; Williams Beuren syndrome-14) is required for the expression of several glycolytic and lipogenic genes in the liver but its role in the pancreatic β-cell is less well-understood. We show here that ChREBP binds directly to the promoter region of the liver-type pyruvate kinase (L-PK) gene in MIN6 β-cells and is required for transcriptional activation by glucose. By contrast, ChREBP regulates the expression of pancreatic duodenum homeobox-1 (*pdx*-1) gene expression in this cell type by an apparently indirect mechanism associated with changes in triglyceride level.
Materials and Methods: cDNA encoding mouse ChREBP was cloned from a MIN6 β-cell library and subcloned into plasmid pcDNA3 (Invitrogen). Polyclonal anti-ChREBP antibody was generated in rabbits immunized with a ChREBP C-terminal peptide conjugated to keyhole Limpit haemocyanin. Promoter activity assessed by single cell microinjection into MIN6 cells (passages #21 -30) of L-PK (-183 -+10 bp) or *pdx*-1 (-2715–0 bp) luciferase reporter constructs, by photon counting imaging normalised to the activity of a co-microinjected CMV.*Renilla* luciferase construct. mRNA levels were determined by quantitative RT-PCR (TaqMan™ or SYBR green). ChREBP binding was assessed by chemical cross linking using formaldehyde and chromatin immunoprecipitation (ChIP), using appropriate PCR primers to amplify ~150 nucleotide regions of genomic DNA. Small interfering (si) RNAs based on nucleotides 508–527 of mouse ChREBP cDNA were synthesised using the Silencer™ siRNA construction kit (Ambion), and introduced with Mirus TransIT™ (Transgenomics) transfection reagent. Insulin secretion was assessed by radio-immunoassay and triglyceride content using a commercial kit (Sigma).
Results: Microinjection of anti-ChREBP antibody completely reversed the 1.67 ± 0.00418 fold activation of the L-PK promoter by 30 *vs* 3 mM glucose. Correspondingly, anti-ChREBP siRNA led to a 87.3 ± 0.25% (n = 3) decrease in ChREBP protein content, a 27.9 ± 1.1% decrease in cellular triglyceride content at 30 mM glucose (vs control), and abolished the increase in endogenous L-PK mRNA induced by 30 mM (vs 3 mM) glucose. Conversely, microinjection of a plasmid expressing wild-type ChREBP augmented L-PK promoter activity at 30 mM glucose by 1.88 ± 0.00472 fold (n >100 from 3 separate preparations) whilst exerting no effect at 3 mM glucose. ChIP assay revealed binding of ChREBP to the previously described carbohydrate response element (paired E-box, L4: $^{-168}$ CACGGGGCACT CCCGTG) of the L-PK promoter. Whereas ChREBP silencing or overexpression caused small but significant increase or decrease respectively in *Pdx-1* mRNA content and promoter activity, no direct binding of ChREBP to any of the ten potential E-boxes in the proximal (2 Kb) *pdx-1* promoter region was detected.
Conclusion: ChREBP confers glucose-responsiveness to the L-PK gene in pancreatic β-cells by binding directly to the carbohydrate response element. By affecting the transcription of L-PK and other lipogenic genes, elevated levels of ChREBP may contribute to triglyceride accumulation in the β-cell during hyperglycaemia. Consequent changes in the expression of the *Pdx-1* and other genes may eventually lead to the loss of normal glucose-stimulated insulin secretion in some forms of type 2 diabetes.

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Gene expression in islet cells

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PPAR-delta is expressed and regulated by cytokines in pancreatic beta-cells

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Background and Aims: Cytokines are major inflammatory mediators of beta-cell dysfunction and death in type 1 diabetes mellitus. Under *in vitro* conditions, beta-cells respond to cytokine exposure by activating various gene networks that alter cellular metabolism, induce chemokine release (and thereby increase insulitis), and cause apoptosis. We have previously shown by microarray analysis that exposure of the beta-cell line INS-1E to IL-1β+IFN-γ induces the transcription factor PPAR-δ and several of its target genes. PPAR-δ, a member of the PPAR nuclear receptor family, controls cellular lipid metabolism. It is also a major regulator of inflammatory responses and atherogenesis through its association with the transcriptional repressor BCL-6 in macrophages. Against this background, the aim of this study was to examine the expression of the PPAR-δ network in cytokine-treated pancreatic beta-cells.
Materials and Methods: FACS-purified primary beta-cells and INS-1E cells were cultured for 6–24 h in the presence or absence of cytokines (IL-1β 50 U/ml + IFN-γ 500 U/ml) or the synthetic PPAR-δ agonist GW501516 (1 μM). Gene expression was analysed by real time PCR and corrected for GAPDH expression. PPAR-δ activation and MCP-1 promoter activity were determined by a luciferase assay using respectively the AoX-luciferase reporter construct containing 3 PPAR-δ response elements and the pMCP-1-514(enh) luciferase construct.
Results: Exposure of FACS-purified primary beta-cells to IL-1β + IFN-γ for 6 h increased by more than 2-fold PPAR-δ mRNA expression (n=7, $p < 0.01$). The expression of the PPAR-δ target genes CD36, adipophilin and acyl CoA synthetase was also increased by 2-fold following exposure to cytokines ($p < 0.05$). A similar induction of the PPAR-δ gene network was observed in INS-1E cells. Cytokines and the synthetic PPAR-δ agonist GW501516 (1 μM) also activated the PPAR-δ nuclear receptor in beta-cells, as assessed by the AoX-luciferase reporter (4-fold increase after 12 h and 2-fold increase after 24 h for cytokines; 2-fold increase after 12 and 24 h for GW501516; n=4, $p < 0.05$ for both). Neither INS-1E nor beta-cells expressed the transcriptional repressor BCL-6, unlike immune cells. As a consequence, PPAR-δ activation by GW501516 (10 h pretreatment) did not decrease cytokine-induced MCP-1 expression or MCP-1 promoter activation (n=3–4), as previously reported for macrophages.
Conclusion: We show for the first time that cytokines activate the PPAR-δ gene network in beta-cells. While this is likely to affect beta-cell lipid metabolism, it does not regulate their pro-inflammatory response, probably because these cells lack the transcriptional repressor BCL-6.
European Foundation for the Study of Diabetes (EFSD)-Johnson & Johnson Programme in Type 2 Diabetes, European Foundation for the Study of Diabetes (EFSD)-Novo Nordisk Programme in Diabetes Research, Fonds National de la Recherche Scientifique, Belgium

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Effects of diazoxide on gene expression in rat pancreatic islets are markedly glucose-dependent and opposite to those of glucose per se

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Background and Aims: Diazoxide exerts beneficial effects on insulin secretion in a setting of long term and continuous hyperglycemia. The molecular basis for such effects is multifactorial with the likely possibility of additional, hitherto unrecognised effects. Here we have used microarray analysis to obtain a full spectrum of effects by diazoxide on the level of gene expression.
Material and Methods: Rat pancreatic islets were cultured overnight at 27 or 5.5 mmol/l of glucose with and without 325 micromolar/l of diazoxide, i.e. during four different conditions. After culture we used Affymetrix technology to run microarray analysis.
Results: The design enabled analysis of dependency and non-dependency of glucose in relation to diazoxide's effects. Altogether 171 genes were up-regulated by diazoxide (signal $\log_2$ ratio > 0.5 i.e. 40% or more) and 208 genes down-regulated (signal $\log_2$ ratio <0.5 i.e. 30% or more). The number of genes (unknown and known) affected by diazoxide were largely (90%) seen only after co-culture with 27 mmol/l glucose. A minority of known genes, which were up-regulated by diazoxide were instead down-regulated by 27 mmo/l of glucose (23%, table). A majority of genes down-regulated by diazoxide were up-regulated by glucose (79%, table). Diazoxide up-regulated genes involved in glycolysis (aldolase b, malic enzyme) and down-regulated genes determining fatty acid oxidation. Diazoxide also down-regulated glutamate decarboxylase and uncoupling protein 2 gene expressions.
Conclusion: The impact of diazoxide on beta-cell metabolism is markedly glucose-dependent. We hypothesize that this dependency is contingent upon effects induced by long-term elevated glucose on glycolysis, mitochondrial metabolism and mitochondrial membrane potential. The glucose-diazoxide interplay of effects may explain the beneficial effects of diazoxide on glucose-induced insulin secretion.

Table: Concordance and discordance between diazoxide and glucose effects (known genes) after 27 mmol/l culture only.

	Upregulated genes >0.5 ($\log_2$)	Downregulated genes < - 0.5 ($\log_2$)
Regulated in parallel with glucose	4	0
Regulated opposite to glucose	11	126
Not affected by glucose	32	34

Data are means of three independent microarray experiments

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Identification of preprodynorphin as a pancreatic gene associated with increased susceptibility to diabetes

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Background and aims: The *Lep^{ob}* and *LepR^{db}* mutations cause more pronounced diabetes, due to β-cell dysfunction, when expressed in mice from the inbred strain C57BLKS compared to the C57BL/6 strain. The C57BLKS strain is also more susceptible to streptoztocin-induced diabetes. The present study was designed to identify differentially expressed islet genes in C57BL/6 and C57BLKS mice and to determine whether one such difference is related to the difference in susceptibility to diabetes.
Materials and methods: Suppression subtractive hybridisation was used to analyse the islets from C57Bl/6 and C57BLKS wild type mice in order to identify genes potentially associated with increased susceptibility to diabetes. The expression levels of the preprodynorphin gene were confirmed in the wild type mice and compared in mutant mice by semi-quantitative RT-PCR. Oral glucose tolerance and intraperitoneal insulin tolerance tests were conducted in prodynorphin knockout mice (provided by Prof. A. Zimmer, University of Bonn, Germany).
Results: A number of potentially relevant genes were identified as differentially expressed between these models. The preprodynorphin gene, which encodes the endogenous opioid precursor prodynorphin, had reduced expression within islets of C57BLKS mice compared to C57Bl/6 mice and was selected for further investigation. Confirmation of differential expression by semi-quantitative RT-PCR showed a threefold reduction in preprodynorphin gene expression in C57BLKS ($p<0.001$) and a fourfold reduction in *LepR^{db}*/ *LepR^{db}* C57BLKS mice ($p=0.0023$) compared to C57BL6 mice. There was no significant change in preprodynorphin gene expression in *Lep^{ob}*/ *Lep^{ob}* compared to wild type C57Bl/6 mice.
Prodynorphin knockout mice had normal fasting blood glucose levels (5.21 ± 0.26 mM) compared with wild type mice (5.11 ± 0.26 mM), but glucose tolerance was impaired (AUC: knockout, 903 ± 77; wild type 708 ± 22 mmol·l^{-1}. 120 min; $p<0.01$). Despite the impaired glucose intolerance in the prodynorphin animals, insulin secretion in response to glucose during the OGTT was not different between the wildtype and knockout mice (AUC for 0–30 min 90.8 ± 20.3 vs. 89.5 ± 16.9ng insulin. 30 min, respectively). There was no significant alteration in insulin tolerance.
Conclusions: The use of suppressive subtraction hybridisation identified the preprodynorphin gene as a differentially expressed gene in C57BLKS compared to C57Bl/6 mice. The preprodynorphin gene regulates beta cell function and a reduction in preprodynorphin gene expression may increase susceptibility to diabetes.

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Cellular mechanism of pancreatic beta cell dysfunction in diabetic *db/db* mice; evidence for deranged gene expression profiles of islet cells obtained by laser capture microdissection

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Background and Aims:The pancreatic islet includes four types of cells that secrete each specific hormone and cooperate each other in the specific architecture. We had established the method to analyze gene expression profiles selectively of pancreatic islet cells by using Laser Capture Microdissection (LCM) and real-time RT-PCR. Our method realizes to obtain the central core and mantle area, and to minimize artificial changes during preparation, as seen in islets isolation. In order to clarify the mechanism of pancreatic beta cell dysfunction in diabetic *db/db* mice, we compared gene expression profiles of pancreatic islet cells in *db/db* mice and lean littermates.
Materials and Methods: The male C57BL/KsJ (*db/db*) and male littermates (*db*/+ and +/+) mice were provided free access to standard feed. Body weight and fasted plasma glucose were measured at the age of eight-week. Pancreatic islet core and mantle area were selectivity obtained by LCM method. For analysis of islet cell gene expression profiling, 25 primer pairs encoding genes associated with pancreatic hormones, cell proliferation, cell differentiation, apoptosis and cell cycle were prepared, and real-time RT-PCR with Sybr Green was applied. Each gene expression was relatively quantified by the comparative Ct method with each result in the +/+ mice as a control.
Results: Body weight of 8-week -old *db/db* mice increased significantly more than other mice (36.6 ± 0.22 vs *db*/+; 24.4 ± 0.26, +/+;17.4 ± 0.2 g, $p<0.0001$), and fasted plasma glucose of *db/db* mice was higher than those in other mice (184.3 ± 31.5 vs 59.3 ± 8.8, 62.7 ± 7.5 mg/dl, $p<0.05$). In the +/+ mice, gene expressions for glucagon, somatostatin and pancreatic polypeptide(PP) in addition to insulin were shown in the mantle area, while the central core showed only insulin 1 and 2 gene expression, suggesting that the central core is consisted only of beta cells. In *db/db* mice, glucagon, somatostatin and PP gene expression were observed in the central core in addition to insulin. A significant increase of insulin 1 and 2 gene expressions was demonstrated in the *db*/+ mice, but not in *db/db* mice (Ins1: 2.12 ± 1.02 and 0.77 ± 0.64, Ins 2: 7.58 ± 3.17 and 1.10 ± 0.32). Gene expressions for cyclin E, caspase 3 and bcl-2 were up-regulated in *db/db* mice more significantly than in other mice. Interestingly, cyclin E gene was expressed only in *db/db* mice. Amylin gene expression was increased more significantly in *db/db* mice than in other mice.
Conclusion: We concluded that LCM method would be a new beneficial tool to analyze the genes expression in the islet cells more specific to the beta cells than isolated islets. In the *db/db* mice, the islet architecture was deranged, and both cell proliferation and apoptosis were readily accelerated at 8 weeks of age. The *db*/+ mice, in spite of heterozygous genetic defect, are not susceptible to diabetes mellitus. The present data suggested a compensatory up-regulation of insulin gene expression in the *db*/+ mice. The further analysis for changes of gene expression profiles in the mice over 10 weeks of age is necessary to elucidate more clearly the mechanism of impaired insulin secretion observed in this mice strain.

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Novel expression of liver FBPase in Langerhans islets of human and rat pancreas

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Background and Aims: It has been postulated that gluconeogenesis in pancreas is irrelevant because insulin release is not stimulated by exogenous lactate and pyruvate. This notion is supported by the absence of PEPCK and Glu-6-Pase expression. This is generally taken to explain why pyruvate is not recycled by gluconeogenesis in β cells and virtually all the pyruvate formed is used by the Krebs cycle. Also, several reports have established the absence of gluconeogenesis in pancreatic islet cells. But, the absence of FBPase expression has not been published elsewhere. Considering the importance of this for pancreatic metabolism, we studied the expression of fructose-1,6-bisphosphatase (FBPase), a key gluconeogenic enzyme, in this organ.
Materials and Methods: Rat and human kidney and pancreas tissues were used to detect FBPase expression by enzymatic activity, immune detection and RT-PCR
Results: We demonstrated that the hepatic FBPase isoenzyme is highly expressed in both human and rat pancreas. Immunolocalization analysis showed that FBPase is expressed in both human and rat Langerhans islets. In humans, FBPase was also located in the canaliculus and acinar cells. Co-localization studies, using anti-insulin and anti-glucagon antibodies, revealed that FBPase is expressed in α and β islet cells. Furthermore, pancreatic FBPase is active and able to sense key regulatory metabolites, such as AMP and fructose-2,6-bisphosphate.
Conclusion: These striking results indicate that FBPase may be a key component of the glucose sensing mechanism that controls the generation of metabolic signals to stimulate the pulsatile and oscillatory release of insulin. These results indicate that FBPase coupled with phosphofructokinase (PFK) may play a crucial role in the metabolism of pancreatic islet cells. The demonstration of gluconeogenic recycling of trioses as a new metabolic signaling pathway may contribute to improving our understanding of the differences between the insulin secretagogues trioses, fructose, and glucose in pancreas.
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The impact of α- to β-cell ratio on *in vitro* and *in vivo* β-cell function

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Background and Aims: In light of the different α-β-cell ratio and distribution in islets of different species, the importance of α-β-cell interaction for β-cell function has been discussed. We have therefore developed an artificial islet system that allows us to study the impact of α-cell mass and α-cell distribution on β-cell function. The first aim was the generation and characterization of a model system based on pseudo-islets consisting of different ratios of GFP- or YCAM2.3-expressing MIN6 β-cells and DsRed-expressing αTC1-9 cells. In addition to microscopic identification of both cell types by fluorescence, β-cell function can be non-invasively assessed by *in vitro* monitoring of up-regulation of insulin promoter-driven GFP expression and increases in intracellular Ca^{2+} by YCAM2.3-FRET in response to glucose stimulation. The second aim was to investigate the impact of α- to β-cell ratio on β-cell function in the transplanted pseudo-islet. Here, we monitored changes in insulin promoter-driven GFP expression or intracellular Ca^{2+} levels in response to stimulation with secretagogues in the transplant *in vivo*.
This study provides insights into the importance of α-β-cell ratio and distribution for β-cell function in artificial islets both *in vitro* and *in vivo.*
Materials and Methods: MIN6 β-cells that stably express either GFP or the Ca^{2+}-sensor YCAM2.3 under the control of the insulin promoter were generated, while αTC1-9 cells were stably transfected with a glucagon promoter-driven DsRed construct. Changes in intracellular Ca^{2+} levels or GFP expression were monitored by imaging either YCAM2.3-FRET or GFP-fluorescence by two-photon laser scanning microscopy (TPLSM). α- to β-cell ratio and distribution were determined by simultaneous imaging of DsRed- versus GFP/YCAM2.3-fluorescence. Pseudo-islets were generated by constant mechanical agitation of a mixture of MIN6-GFP/YCAM2.3 and αTC-DsRed cells for 72 h. These pseudo-islets were used to monitor glucose-stimulated insulin promoter activity and intracellular Ca^{2+} both *in vitro* and *in vivo*, following transplantation under the kidney capsule of immune deficient nude mice. The transplant was allowed to get vascularized for 3–5 weeks. The localization of the transplant under the kidney capsule allowed monitoring of fluorescence by TPLSM *in vivo* in the cannulated and extracted kidney.
Results: We were able to generate artificial islets with various α- to β-cell ratios. Pseudo-islets containing at least 20% α-cells showed a significantly higher glucose-stimulated insulin promoter activity *in vitro.* Pseudo-islets generated from MIN6-YCAM2.3 and αTC-DsRed cells demonstrated changes in intracellular Ca^{2+} subsequent to *in vitro* stimulation with glucose or the sulfonylurea compound tolbutamide that are similar to those observed in mouse islets. The stimulatory effect of tolbutamide has been confirmed *in vivo.*
Conclusion: We were able to create a model system to study the impact of α- to β-cell ratio on secretagogue-stimulated insulin gene transcription and increase in intracellular Ca^{2+} both *in vitro* and *in vivo.*
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Regulation of glucagon gene expression by insulin is abolished after FOXO1 silencing

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Background and Aims: Whilst the winged-helix transcription factor FOXO1 has been suggested to play an important role in pancreatic β-cell development and function, a role for FOXO1 in the pancreatic α-cell has not previously been described. Here, we use RNA silencing to determine the role of FOXO1 in the regulation of preproglucagon gene expression and glucagon secretion in clonal αTC1-9 cells.
Materials and Methods: αTC1-9 cells were maintained in Dulbecco's Modified Eagle's Medium containing 17 mM glucose, 10% foetal bovine serum, penicillin (100 IU/ml) and streptomycin (100 mg/ml). An siRNA duplex against FOXO1 and a scrambled (Sc) RNA duplex were constructed using the Ambion Silencer™ kit and introduced into cells by incubation for 48 h in the presence of *Trans*IT-TKO transfection reagent (Mirus®). Immunocytochemical analysis was performed on methanol-fixed cells using a primary anti-FOXO1 polyclonal antibody (Cell Signalling) and FITC-labelled anti-rabbit secondary antibody (Jackson) using a Leica TCS-NT inverted optics microscope (x63 objective lens). Fluorescence intensity was quantified off-line with Volocity™ software. Real-time PCR analysis was performed using an Opticon 2 (M J Research) cycler. Glucagon secretion was measured following a 1 h pre-incubation in Krebs Ringer Buffer (KRB), followed by subsequent incubation for 30 min with additions as given. Secreted and total glucagon were measured by radioimmunoassay (Linco). Student's t-test and one-way ANOVA analysis were performed in Excel and Graphpad™ Prism software, respectively.
Results: Immunocytochemical analysis of αTC1-9 cells showed the presence of endogenous FOXO1 immunoreactivity largely within the nuclear region after culture at either high (30 mM) or low (0.5 mM) glucose concentrations. Stimulation of cells for 6 h with 17nM insulin resulted in a significant translocation of FOXO1 to the cytosol and cell periphery such that the ratio of cytosolic: nuclear immunoreactivity increased from 13.3 ± 2.7% to 39.0 ± 4.0% ($p < 0.005$; n = 3 separate experiments). Real time quantitative RT-PCR also revealed a 28 ± 0.005% decrease in preproglucagon mRNA ($p < 0.05$) in insulin-treated cells. In the absence of insulin, transfection of cells with anti-FOXO1 siRNA duplex decreased detectable FOXO1 immunoreactivity by 86 ± 2.8% ($p < 0.05$) and decreased the level of preproglucagon mRNA by 58.0 ± 0.07% ($p < 0.005$) of control (ScRNA treatment) levels. Whereas insulin caused a 28 ± 0.005% decrease ($p < 0.05$) in preproglucagon mRNA levels in control cells, the hormone exerted no further effect in FOXO1- deleted cells. Finally, acute treatment with 17nM insulin inhibited glucagon secretion in control (ScRNA treated) cells by 20 ± 0.055% ($p = 0.002$), whilst having no significant effect in cells transfected with the anti-FOXO1 SiRNA duplex.
Conclusion: We conclude that FOXO1 plays an important role in the regulation by insulin of preproglucagon gene expression and secretion in pancreatic α-cells.
Support: JDRF and Wellcome Trust

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Signal transduction in islet cells I

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Enhanced activation of PI3K signaling pathway by IGF-1 and glucose contributes to increase beta cell mass in undernourished fetal rats

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Background and Aims: We have previously shown that Wistar fetuses from protein-caloric undernourished (U) pregnant rats at 21.5 days post coitum (dpc) exhibit increased beta cell mass. This effect has been recently related to a higher stimulation of beta cell replication due to an increased in both locally IGF-1 and glucose oxidation in beta cell in U fetuses. The mitogenic IGF-1 action is mediated by the IGF-1 receptor (IGF-1R) and via insulin receptor substrate (IRS)-2. IRS-2 engages at least two distant signaling pathways, the phosphatidylinositol 3-kinase (PI3K) pathway and mitogen-activated protein kinase (MAPK) pathway. The IGF-1-induced mitogenic response is glucose dependent and glucose itself can stimulate beta cell mitogenesis by activating signals downstream of IRS-2, like Erk-1/2 and p70S6K. The aim of the present study was to characterize the intracellular glucose and IGF-1-mediated signal transduction pathway(s) that can be involved in the increased beta cell mass found in U fetuses. To this end, we evaluated in control (C) and U fetal pancreatic islets: 1) beta cell proliferation induced by glucose and IGF-1, 2) IGF-1R protein content and its tyrosine kinase activity upon IGF-1 stimulation and 3) protein content and phosphorylation of proteins involved in the PI3K and MAPK pathways upon glucose and IGF-1 stimulation.
Materials and Methods: We used fetuses proceeding from mothers food restricted (65% of food intake) during the last week of gestation and their respective controls. Islets were isolated at 21.5 days of gestation and cultured during 2 days. After a period of quiescence (serum-starved) islets were incubated with basal (3 mM) or stimulatory (17 mM) concentrations of glucose±IGF-1 (100ng/ml) at 5, 10 and 15 minutes. At the end of the culture period, cells were lysed, immunoprecipitated and immunoblotted with specific antibodies. Incorporation of BrdU was used as indicator of cell proliferation.
Results: Islets from U fetuses showed an increase in the content of IGF-1R. Upon IGF-1 stimulation, thyrosine kinase autophosphorylation of IGF-1R and thyrosine phosphorilation of IRS-2 was higher in U fetal islets. Similarly, phosphorylation of p70S6K by glucose and IGF-1 was augmented in undernourished animals although the total protein content remained unchanged. However, Erk-1/2 protein content and phosphorylation were unaltered in response to glucose and IGF-1. In addition, U fetuses showed a higher mitogenic response to glucose and IGF-1 than C fetuses.
Conclusion: This study points out that: 1) several elements of the mitogenic signal transduction pathway are present in pancreatic fetal islets and can be stimulated by glucose and IGF-1, 2) food restriction of the mother increases in its fetuses the content of IGF-1R and the phosphorylation of IGF-1R and IRS-2 which are critical to the control of beta cell survival, 3) the increased IGF-1 and glucose sensitivity seems to be mediated via IRS-2/PKB/p70S6K and 4) this specific activation may have an important role in increasing the islet mass in U fetuses.

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Increased cell proliferation induced by the protein p8 in INS-1 beta-cells via activation of the phosphatidylinositol-3'-kinase and MAP kinase pathways

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Background and Aims: Overexpression of the stress protein p8 is known to increase glucose dependent cell proliferation in insulin producing cells. In this study we investigated p8 associated intracellular signal transduction in the glucose dependent beta cell line INS-1.
Methods and Results: INS-1 beta cells stably overexpressing p8 (p8-INS-1) displayed a 6fold increase in p8-mRNA levels and a 2fold increase in cell proliferation as compared to wild type INS-1 beta cells. Cell proliferation was measured using [^{3}H]-thymidine incorporation. Activation of mitogenic signaling proteins was assessed using co-immunoprecipitation and immunoblot analysis. p8 protein is ubiquitinated and degraded by the proteasome pathway. Inhibition of proteasome dependent protein degradation by lactacystin resulted in increased cell proliferation in the presence of low glucose concentrations (0–6 mM). p8 dependent cell proliferation seems to

be mediated via the phosphatidylinositol-3'-kinase (PI3K) pathway and protein kinase C (PKC), because coimmunoprecipitation demonstrated protein binding of both PI3K and PKCzeta to p8. Inhibition of PKCzeta resulted in complete inhibition of cell proliferation in INS-1 beta cells. In contrast, inhibition of PI3K lead only to partial reduction in cell proliferation suggesting PI3K independent activation of PKCzeta in p8 overexpressing cells. Further, protein association of p8 to MAPK (erk1/2) was observed, whereas no association of p8 to p38MAPK could be detected.
Conclusion: These results suggest that p8 is an important protein involved in cell proliferation in insulin producing cells. p8 effects are dependent on ubiquitination and proteasomal degradation of the protein and are mediated via PI3K, PKCzeta and MAPK dependent signaling pathways. These results yield insight into the regulation of nutrient dependent cell proliferation in pancreatic beta cells.
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The calcium-sensing receptor regulates beta-cell proliferation through activation of ERK1/2
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Background and Aims: The extracellular calcium-sensing receptor (CaR) is expressed on pancreatic β-cells, and we have demonstrated recently that activation of the CaR enhances insulin secretion from β-cell lines and from human islets of Langerhans. In other tissues, CaR activation is often associated with increased cell proliferation. We have now used a calcimimetic CaR agonist to investigate the effects of CaR activation on β-cell proliferation. Mitogen activated protein kinases have been implicated in β-cell proliferative responses, so we have determined whether the CaR influences β-cell function through the activation of the ERK1/2 cascade.
Materials and Methods: The rate of proliferation of mouse insulin-secreting MIN6 cells was measured by bromo-deoxyuridine (BrdU) incorporation into DNA using a colorimetric immunoassay. Apoptosis was assessed by measuring DNA fragmentation on ethidium bromide-stained agarose gels. Cisplatin (100 μM, 24 h) was used to induce apoptosis. The expression and activation of ERK1/2 were measured by immunoblotting using antibodies that detected either total ERK1/2 immunoreactivity, or the phosphorylated activated form of the enzymes. ERK1/2 activity was inhibited using PD98059 (50 μM). Cells were serum-starved for 24 h prior to experiments.
Results: The CaR agonist A-568 acts to sensitize the CaR to activation by extracellular Ca^{2+} ($[Ca^{2+}]_e$). Consistent with this, in the absence of $[Ca^{2+}]_e$ A-568 (1 μM) had no detectable effect on BrdU incorporation. However, in the presence of $[Ca^{2+}]_e$ (0.25–2.25 mM), A-568 (1 μM) enhanced BrdU incorporation to a similar extent as 10% fetal calf serum (FCS) replacement (A-568, 2.25 mM $[Ca^{2+}]_e$, 290 ± 19% basal, mean±SEM, n=8, $P<0.01$; FCS, 257 ± 23% $P<0.01$), consistent with CaR activation stimulating proliferation of MIN6 cells. The proliferative effects of A-568 (0.01–1 μM) on MIN6 cells were not additive to those of 10% FCS alone ($P>0.2$ at all concentrations). Expression of the 42 kDa and 44 kDa ERK1/2 proteins was not affected by CaR activation, but measurements of the phosphorylated activated ERK1/2 showed a small increase in activation of both isoforms in response to A-568 (1 μM) alone, that was greatly enhanced by the presence of $[Ca^{2+}]_e$. CaR activation by A-568 had no detectable effect on basal DNA-laddering but enhanced cisplatin-induced DNA-laddering, whereas this was reduced by the presence of insulin (5 μM), suggesting that CaR activation in MIN6 cells is pro-apoptotic rather than protective.
Conclusion: These results demonstrate that CaR activation stimulates proliferation of MIN6 cells, and implicate the ERK1/2 signalling pathways in the proliferative response. The regulation by CaR activation of both secretory and proliferative responses suggests that signalling through the CaR may play an important regulatory role in normal β-cell function.

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Gene expression profile of tungstate-induced beta cell mass increase in STZ rats
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Background and Aims: It is well demonstrated that in the beta cell population of the pancreas there is a dynamic turnover, which results from the net balance of several processes; beta cell replication, apoptosis and neogenesis. These processes have been studied in partial pancreatectomy and glucagon-like peptide 1 treated animals, where an increase in pancreas regeneration has been observed. Similarly, sodium tungstate, which decreases hyperglycemia in several animal models of diabetes, promotes a rise in the beta cell mass of nSTZ and STZ animals. However, the molecular mechanisms underlying this pancreas regeneration remain unknown. Therefore the objective of this study is to identify which genes are up or down regulated in the increase of the beta cell population of STZ rats treated with sodium tungstate.
Materials and Methods: STZ-rats and healthy rats were treated with tungstate and at the end of the treatment the animals were sacrificed and pancreatic RNA isolated. Three chips (Affymetrix RAE-230A) were hybridized for each of the four experimental groups (untreated and treated healthy rats and untreated and treated diabetic rats). The raw intensity data obtained from the microarrays was normalized and summarized using the Bioconductor package RMA. Moreover differential expression between the different groups was assessed by linear models with a Bayesian approximation with the Bioconductor package LIMMA, obtaining a list of significant genes differentially expressed due to diabetes, treatment and the combination of both. The genes significantly activated or inhibited by the treatment were clustered using dChip and organized with NetAffx Gene Ontology Mining Tool. Some of these changes were checked by quantitative RT-PCR, immunohistochemistry and measurement of the protein levels in serum. Furthermore, pancreatic RNA isolated from phlorizin treated rats and quantitative RT-PCR was used to asses which genes changed due to the reduction of the glycemia and which were a direct target of tungstate.
Results: Firstly, the expression levels of many genes involved in several pathways (lipid and glucose metabolism, endocrine and exocrine enzymes, etc.) were modified in the STZ untreated rats. Secondly, sodium tungstate in the diabetic animals increased the expression of endocrine (insulin) and exocrine (amylase) genes and decreased the expression of genes involved in lipid metabolism (HMG-CoA synthase, acetil CoA dehydrogenase). It is worth noting that we also observed an increase in several genes involved in proliferation and differentiation processes (TGF-β3, RKIP, trefoil factor 2, etc.) in tungstate-treated diabetic animals. At last, no significant differences in gene expression were observed between untreated and treated healthy rats, supporting our previous data that tungstate administration does not exert any effect on glucose metabolism of healthy animals.
Conclusion: Sodium tungstate activates various pathways which control the pancreatic plasticity, resulting in an increase of the beta cell mass. This effect together with extra-pancreatic effects leads to the decrease of the glycemia.
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Glucagon-like peptide-1 activates MAPK by an interaction of GTPase Rap with CRKII and C3G in INS-1E insulinoma cells
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Background and Aims: Glucose and the incretin hormone glucagon-like peptide-1 (GLP-1) regulate numerous beta-cell functions such as insulin secretion, insulin biosynthesis and proliferation in a synergistic manner. We previously demonstrated a central role for the small G-protein Rap in the regulation of synergistic signal transduction of GLP-1 and glucose. We now examined regulation and signal transduction by Rap in INS-1E insulinoma cells.
Materials and Methods: INS-1E cells were stimulated with 10 nM GLP-1 at low (2.5 mM) and high (15 mM) glucose concentration. Subsequently, differential immunoblots, MAPK and PI3K assays were performed.
Results: Rap was associated with tyrosine-phosphorylated IRS-2 and p85alpha (the regulatory subunit of PI3K) in INS-1E cells stimulated with high glucose and GLP-1. Interestingly, we identified a tyrosine-phosphorylated protein of 42 kDa as CRKII, which interacted with Rap in a glucose-

dependent manner. Rap also associated with the G-protein activating guanine-nucleotide exchange factor C3G at high glucose. A negative dominant C3G fragment, which interfered with the interaction of CRKII with C3G, inhibited MAPK activation by glucose and GLP-1. These results indicate that C3G and CRKII regulate glucose and GLP-1 induced activation of Rap in beta-cells. In addition, Rap activated PI3K by interacting with tyrosine-phosphorylated p85alpha.
Conclusion: Rap is a central glucose-dependent effector of MAPK and PI3K signaling in beta-cells and thus a molecular target for pharmacological induced therapeutic beta-cell growth in diabetes mellitus.

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Protein profiling of INS1E cells by two-dimensional gel electrophoresis and mass spectrometry
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Background and Aims: The rat insulinoma-derived clonal β-cell line INS1E, is widely used as a suitable model for studying the stimulus-secretion coupling in pancreatic β-cells. Our aim was to establish a reference protein map of INS1E cells for comparative studies concerning global protein profiles, which will contribute towards better understanding of the mechanism of insulin secretion.
Materials and Methods: INS1E cells were cultured in RPMI 1640 containing 11 mM glucose and supplemented with 10% FCS. Proteins were extracted from $15x10^6$ cells and solubilized in modified lysis buffer. Subsequently, proteins were separated by two-dimensional gel electrophoresis (2-DE) using an optimized protocol where iso-electric focusing was performed on non-linear, immobilized pH gradient strips (pH 3–10) and the second dimension on 8–16% gradient SDS gels. The separated proteins were visualized by colloidal Coomassie stain and image analysis was performed by PDQuest. Selected protein spots were identified by matrix assisted laser desorption/ionization time of flight mass spectrometry.
Results: When 2-DE was performed on protein samples from INS1E cells highly reproducible (n=4) gel images were obtained containing 686 valid spots, which remained after removal of artefacts. The correlation coefficient among the gels was 0.75. When comparing the protein spots on the gels from INS1E cells with that of mouse islets, the high abundant and most distinct proteins, such as, GRP78, VCP, TRA1, TUB, ACT, HSPs, GRP58, GDI1 and MDH1 appeared with a similar pattern. Interestingly, one of the spots representing protein disulfide isomerase was under-expressed in the INS1E cells, similar findings in the cultured mouse islets. However, spot positions corresponding to the intermediate filaments were over-expressed in the INS1E cells. Whereas, exocrine proteins appeared as distinct spots in the islet protein map, they were absent in the INS1E cell protein maps. Signalling proteins, like 14-3-3 proteins and antioxidants, e. g., SOD were highly expressed in INS1E protein maps.
Conclusion: The identified proteins contribute to structural and functional profile of the INS1E proteome and this first-draft reference map will serve as an important tool for future studies of differential protein expression related to pancreatic β-cell function.

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Proteomic approach to search for proteins involved in the mitogenic effect of recombinant human prolactin in primary cultures of human pancreatic islets
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Background and Aims: Transplantation of isolated pancreatic islets from cadaveric organ donors is a promising alternative for treatment of type 1 diabetes, however, this approach is severely limited by the shortage of organ donors. *Ex-vivo* islet cell culture prior to transplantation appears as an attractive alternative, however, maintenance of human islets in culture has been a difficult task. Therefore, stimulation of islet cell proliferation and differentiation *in vitro* constitutes a major scientific and clinical challenge. Previous results from our laboratory have demonstrated a significant beneficial effect of rhPRL treatment on cell proliferation and secretory function of human islet primary cultures. In order to probe into the molecular events involved in the intracellular action of rhPRL in human pancreatic islets, we set out to identify proteins with altered expression levels upon rhPRL cell treatment, using 2-dimensional (2D) gel electrophoresis and mass spectrometry (MS).
Materials and Methods: Human pancreatic islets were isolated after ductal distension of the pancreas and tissue digestion with Liberase HI, following the automated method of Riccordi et al, with modifications. After isolation, the islets cultures were stimulated for 96 h with either rhPRL or vehicle. Total protein extracts were then subjected to proteomic analysis using 2D gel electrophoresis and MALDI-TOF MS.
Results: In order to describe the changes in protein expression, we performed 2D gel electrophoresis of total protein extracts from primary cultures derived from four different human islet preparations incubated in the presence or in the absence of rhPRL. An average of 300 different protein spots were obtained, 16 of which were modified (cut-off: 2 fold) upon rhPRL treatment. Four of these were successfully identified using peptide-mass-fingerprinting. Nine non-regulated protein spots were also chosen as internal controls, three of which were identified. The proteins identified were grouped according to their biological functions (heatshock, ionic trafficking, structural).
Conclusion: Our study provides, for the fist time, important proteomic information towards understanding the molecular mechanisms involved in the action of PRL in human pancreatic islets. The new approach presented here may be useful to reveal relevant molecular mechanisms involved in PRL action on human pancreatic islets, and facilitate identification of new and specific molecular targets involved in islet cell function and proliferation.

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Calsenilin/DREAM/KChIP3 regulates islet glucagon release and prodynorphin expression
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Background and Aims: Calcium-binding proteins regulate transcription and secretion of pancreatic islet hormones. Here we characterize the expression and function of the calcium-binding protein KChIP3 in neuroendocrine cells.
Materials and Methods: Mouse islets were assayed for KChIP3 transcript abundance using quantitative real time PCR with SybrGreen detection and for protein using western and immunohistochemical detection with antibodies specific to the KChIP3 protein. Islets hormone secretion from KChIP3 knockout and control mice was determined through radioimmunoassay. The role of KChIP3 on transcription was assessed through luciferase reporters and quantitative real time PCR and electrophoretic mobility shifts were employed to assay alpha and beta cell KChIP3 DNA complexes.
Results: KChIP3 message and protein were detected in mouse islets, human islets, the beta cell line Min6, and the alpha cell line AlphaTC1. KChIP3–/– mice had an increased rebound in blood glucose following insulin-induced hypoglycemia, and isolated KChIP3–/– islets had elevated glucagon secretion (~30%) in low glucose compared with controls (n=8 $p<0.05$). Since KChIP3 regulates neuronal dynorphin expression and dynorphin is produced in beta cells, we determined whether this pathway is affected in KChIP3–/– islets. Upon glucose stimulation KChIP3–/– and control islets increased prodynorphin transcription, while in KChIP3–/– islets message levels were elevated ~2-fold at low (2 mM) glucose concentrations (n=5 $p<0.05$). In Min6 cell extracts, KChIP3 interacted with the prodynorphin promoter. The endogenous prodynorphin message and a reporter construct containing the prodynorphin 5' flanking region showed higher expression levels in Min6 cells upon glucose stimulation (n=10 $p<0.05$).
Conclusion: These results suggest KChIP3 participates in intraislet dynorphin regulation of glucagon secretion, and provides a molecular basis for opiate stimulation of glucagon secretion first observed over 25 years ago.
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PS 24
Signal transduction in islet cells II

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Modulation of pancreatic islet and hepatocyte function with a novel glucokinase activator

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Background and Aims: The glucose-sensing enzyme glucokinase (GK) plays a key role in glucose metabolism. Activation of GK at elevated glucose levels produces a rise in insulin secretion from the pancreas, as well as stimulation of glucose uptake in liver, the combination of which leads to an increase in glucose disposal and the return of blood sugar to basal levels. Recently, small molecules that activate GK via binding to an allosteric site on the enzyme have been discovered. Through their ability to enhance the activity of GK, such GK activators hold promise as a novel and effective treatment for type 2 diabetes. Herein we describe the effects of a novel GK activator in pancreatic beta-cells and hepatocytes.
Materials and Methods: An enzymatic GK assay using purified recombinant human islet GK was used to evaluate the effects of a small molecule activator. Insulin secretion was measured in isolated rat pancreatic islets. Glucose uptake in cultured rat hepatocytes was assessed with 3H-2-deoxy-D-glucose. Oral glucose tolerance test (OGTT) was performed in healthy conscious Wistar male rats.
Results: In search of GK activators, we have identified a novel compound, LY2121260, which increased GK activity. 10 µM LY2121260 increased the enzyme Vmax by 40% and decreased glucose S0.5 from 6.8 ± 0.5 to 0.4 ± 0.1 mM. Incubation of rat pancreatic islets with different concentrations of LY2121260 produced a dose-dependent increase in glucose-induced insulin secretion (EC50=0.2 ± 0.1 µM). The activator was found to stimulate insulin secretion at intermediate glucose concentrations. Maximal stimulation of insulin secretion (4-fold) with LY2121260 was observed at 8 mM glucose. In cultured rat hepatocytes, LY2121260 enhanced glucose uptake by 140% with EC50=1.7 ± 0.4 µM. And finally in OGTT in healthy rats, LY2121260 dose-dependently reduced increases in blood glucose levels after glucose challenge. Treatment with 50 mg/kg LY2121260 resulted in 28% reduction in glucose exposure compared to control.
Conclusion: Results of the study support the concept that GK activators represent a new class of compounds that increase both insulin secretion and hepatic glucose utilization, and in so doing may prove to be effective agents for the control of blood glucose levels in patients with type 2 diabetes.

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Effects of anti-diabetic drugs on beta cell glucose metabolism in a clonal cell line, BRIN-BD11

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Background and Aims: Previous studies have shown that prolonged exposure of pancreatic beta cells to sulphonylurea drugs results in desensitization to subsequent insulinotropic stimuli.The mechanisms by which desensitisation occurs are unknown. The current study aimed to probe the effect of prolonged exposure to sulphonylureas on glucose metabolism.
Materials and Methods: BRIN-BD11 cells were incubated for a period of 18 h in the presence or absence of tolbutamide (100 µM) or glybencamide (1 µM). This was followed by a period of incubation in the presence of [U-^{13}C]glucose. The resulting glucose derived metabolites were extracted using a perchloric acid extraction procedure. The neutralised supernatant was prepared for NMR analysis. ^{13}C NMR spectra were acquired on a Bruker DRX 500 spectrometer.
Results: Preincubation in the presence of tolbutamide or glybencamide for a period of 18 h did not alter the amount of labelled glutamate, alanine or lactate produced from ^{13}C labelled glucose (major metabolic end-products). However, analysis of the isotopomers formed showed that the ratio of the flux through pyruvate carboxylase to the flux through pyruvate dehydrogenase (pc/pdh) was significantly reduced ($p < 0.05$) in the presence of tolbutamide (from 0.32 ± 0.01 to 0.24 ± 0.02). Under control conditions the amount of glucose remaining in the medium was 19.5 nmol/mg protein. Preincubation in the presence of tolbutamide did not significantly alter this value.
Preliminary studies using ^{31}P NMR of intact living cells in a bioreactor system showed that the drug did not alter the ATP levels significantly during prolonged exposure.
Conclusions: The present study demonstrates that prolonged beta cell exposure to tolbutamide resulted in changes in flux through key enzymes involved in glucose metabolism. Alteration of the PC and PDH fluxes may have important consequences for insulin secretion, as these enzymes will determine the rate of oxidation of glucose.
Support: The HRB Ireland are acknowledged for funding.

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Autocrine signaling of insulin mediates 3-phosphorylated inositol lipid generation following glucose stimulation

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Background and Aims: Phosphatidylinositol 3-kinases (PI3Ks) have a central role in pancreatic β-cell function. Downstream events include the regulation of K_{ATP} channel activity, insulin secretion and gene transcription. Little data is available on the 3-phosphorylated inositol lipids (3-PI's) that are the products of these kinases.
Materials and Methods: High-performance liquid chromatography was used to seperate ^{3}H-labeled inositol lipids extracted from HIT-T15 cells and mouse pancreatic islets.
Results: We characterized these PI3K-products in insulin secreting HIT-T15 cells and were able to demonstrate for the first time the presence of phosphatidylinositol 3,5-bisphosphate (PtdIns(3,5)P_2). We then showed that glucose alone can significantly increase PtdIns(3,4,5)P_3, PtdIns(3,4)P_2 and notably PtdIns(3,5)P_2. We investigated the mechanism(s) whereby glucose-stimulation is able to generate these molecules and found that a substantial proportion of the glucose-stimulated increase in PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2, but not PtdIns(3,5)P_2 was secondary to the influx of Ca^{2+} through voltage-dependent L-type Ca^{2+} channels. These channels are the principal driving force leading to insulin secretion and thus formation of these lipids is likely to be secondary to insulin exocytosis. This was confirmed as blockade of the β-cell insulin receptor completely abrogated the glucose-mediated increase of all 3 lipids, driving their concentrations below basal levels. This 3-phosphorylated inositol lipid generating pathway were verified in mouse pancreatic islets.
Conclusion: These data suggest that glucose-induced increases in 3-PI's are principally involved in regulating events occurring after initial secretion and are not driving primary events in β-cell stimulus-secretion coupling. An additional important observation was that secreted insulin is required to sustain the basal levels of these important signaling molecules.

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Blockade of integrin β1-laminin 5 interaction affects spreading and insulin secretion of rat beta-cells attached on extracellular matrix

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Background and Aims: When attached on a matrix produced by a rat bladder carcinoma cell line (804G matrix), rat pancreatic beta-cells spread in response to glucose and secrete more insulin compared to cells attached on poly-L-lysine (PLL). The purpose of this study was to demonstrate, using blocking antibodies, that this effect is mediated by the interaction between β1-integrin expressed on beta-cells and laminin 5 (LN5) present in 804G matrix.
Materials and Methods: FACS-sorted rat pancreatic beta-cells were plated on either PLL or 804G matrix. Cells were incubated under control conditions or in presence of blocking antibodies raised against β1-integrin, LN5 and fibronectin (FN). Non immune mouse, rabbit and hamster Ig were used as control. Cells were analysed for attachment and spreading under an inverted microscope. Spreading was quantified by measuring the area profile of cells with ScionImage™ Software. Insulin secreted from 2×10^4 beta-cells was measured after a static incubation test (1 h basal and 1 h stimulated) by radioimmunoassay and expressed in % of insulin content. Single cell secretion was assessed by reverse haemolytic plaque assay (RHPA), and insulin release was expressed as total plaque area/100 cells. Data are presented as mean ± SEM of 3 or more independent experiments. Levels of significance for differences between groups were evaluated using Student's unpaired t-test, with $p<0.05$ considered significant.

Results: Anti-LN5 antibody, but not anti-FN antibody, inhibited spreading of beta-cells attached on 804G matrix. These antibodies did not affect attachment of beta-cells on PLL (n=3). Under control conditions, insulin secreted by beta-cells attached on PLL was 0.38 ± 0.13% and 3.86 ± 0.37% at 2.8 and 16.7 mM glucose, respectively. 804G matrix increased insulin secreted at 16.7 mM glucose (7.67 ± 0.90%, p=0.02). In the presence of anti-LN5 antibody, stimulated insulin secretion from beta cells attached on 804G matrix was reduced (3.23 ± 0.49%, p=0.012) and was similar to insulin secreted from cells attached on PLL. Anti-FN antibody and control Ig did not affect either basal or stimulated insulin secretion. When expression of the two well-known LN5 ligands, β1- and β4-integrins, was assessed by western blotting and RT-PCR, only β1-integrin was detected in beta-cells. On 804G matrix, anti-β1-integrin antibody reduced area profile of beta-cells compared to control (0.084 ± 0.011 vs 0.124 ± 0.007 arbitrary units; p=0.027). Stimulated insulin secretion was 2 ± 0.3% in control cells plated on 804G and 1 ± 0.1% in cells incubated with anti-β1-integrin antibody (p=0.016). RHPA showed similar results: after stimulation, total plaque area (arbitrary units) was reduced in the presence of anti-β1-integrin antibody compared to control (123.8 ± 10.6 vs 383.5 ± 45.9, p=0.0015). Control Ig did not affect either spreading or insulin secretion.
Conclusion: Anti-β1-integrin and -LN5 antibodies interfere with spreading of beta-cells resulting in decreased insulin secretion in response to glucose. It is concluded that outside-in signalling via engagement of β1 integrins by LN5 could be an important component of normal beta-cell function.
Support: Swiss National Science Foundation

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Molecular characterization of the interactions between IA-2 and the PDZ domains of proteins in the beta cell
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Background and Aims: IA-2, a member of the tyrosine phosphatase family, is a major autoantigen of type 1 diabetes since autoantibodies to IA-2 appear in most patients several years before clinical onset. We previously showed that the juxtamembrane domain is the target of autoantibodies in most sera of type 1 diabetic patients. Nevertheless, the function of this autoantigen in the beta cell is to date unknown. The aim of the present study was to characterize the sites of interaction between IA-2 and two other β cell proteins in an attempt to understand its function and possible role in the physiopathology of diabetes.
Materials and Methods: Several recombinant protein domains were produced in a baculovirus/insect cell system and in *E. coli*. These proteins included the intracytoplasmic (AA 601-979), the juxtamembrane (AA 601-700) and the PTP (AA 701-979) domains of IA-2, the PDZ domain of nNOS and the PDZ domain of β2-syntrophin. We first investigated by ELISA the binding of the PDZ domains to the juxtamembrane and PTP regions of IA-2. Next, we localized the interaction sites by the Spot technique: the entire sequence of IA-2 was synthesized on cellulose membranes in the form of 479 overlapping peptides (25 mer, frameshifted by two amino acids), and then the PDZ domains were allowed to react with these peptides. These results were confirmed in an inhibition ELISA format with anti-IA-2 antibodies. Reciprocally, the critical residues of the PDZ domains implicated in the binding to the IA-2 were also identified by the Spot technique.
Results: By ELISA we showed that the binding of nNOS and β2-syntrophin to IA-2 required the PDZ domains of these proteins. Peptide scanning experiments also demonstrated that the consensus sequences ^{27}GLGF^{30} and ^{125}GLGI^{128} (in nNOS and β2-syntrophin PDZ, respectively) were implicated in the binding to IA-2, as described in the classical PDZ interactions. Concerning the binding of IA-2 to nNOS, we showed by Spot technique that residues ^{639}KSLF^{642} in the juxtamembrane domain of IA-2 were crucial for this interaction. These results were confirmed in an inhibition ELISA format, where the binding of the nNOS PDZ to IA-2 was specifically inhibited by anti-IA-2 antibodies. Five anti-IA-2 antibodies recognizing the juxtamembrane or PTP domain were tested: only the anti-juxtamembrane domain antibodies were able to inhibit the binding of the nNOS PDZ to IA-2. On the other hand, the binding of β2-syntrophin to IA-2 required a region in the carboxy-terminus of both the juxtamembrane and the PTP domains. Moreover, we confirmed the importance of phosphorylation of the β2-syntrophin PDZ and especially the presence of phosphorylated serine 129, which improved the interaction with IA-2.
Conclusion: Through its interactions with nNOS and β2-syntrophin, IA-2 could be implicated in the signaling pathways involving nitric oxide and could link secretory granules to the actin cytoskeleton of the beta cell. We showed that the PDZ domains of both proteins were sufficient for their binding to IA-2. These interactions involved the consensus sequences GLGF/I within the classical PDZ domains. The characterization of these interactions might be useful for molecular modelization of the IA-2 protein. In particular, the juxtamembrane domain could be modeled as an atypical PDZ domain.
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Diacylglycerol lipase and cannabinoid receptor expression and function in pancreatic beta-cells
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Background and Aims: The pancreas contains high levels of mRNA coding for diacylglycerol lipase (DAG lipase), an enzyme that catalyses the conversion of DAG to 2-arachidonylglycerol (2-AG). 2-AG is an endocannabinoid that activates CB1 receptors, and it has been shown to elevate intracellular calcium ($[Ca^{2+}]_i$) in neural cells. Since elevations in $[Ca^{2+}]_i$ play a key role in the regulation of insulin secretion the aims of the current study were first to determine whether pancreatic β-cells express DAG lipase and CB1 receptors, and then to measure the effects on β-cell $[Ca^{2+}]_i$ and on insulin secretion of the pharmacological modulation of CB1 receptor activity.
Materials and Methods: RT-PCR using primers directed against DAG lipase and CB1 receptors were performed on mRNA isolated from MIN6 insulin-secreting cells and protein expression was determined by immunohistochemistry and by PAGE and Western blotting. The role played by the CB1 receptor signal transduction cascade in insulin secretion was investigated using a CB1 receptor agonist (WIN55,212-2) and antagonist (AM251) in perifusion experiments with MIN6 cells configured as three-dimensional pseudoislets. The effects of CB1 receptor activation on $[Ca^{2+}]_i$ were determined by single cell microfluorimetry of Fura 2-loaded MIN6 cells.
Results: Pancreatic β-cells expressed elements of the CB1 receptor cascade. Thus, mRNAs coding for the α and β isoforms of DAG lipase were expressed in MIN6 cells, and immunohistochemical analyses of rat pancreatic sections indicated that both DAG lipase isoforms were localised to the islets of Langerhans. RT-PCR and Western blotting indicated that CB1 receptor mRNA and protein were expressed in MIN6 cells, as well as in rat islets and exocrine pancreas. Activation of CB1 receptors with WIN55,212-2 (1–5 μM) caused maintained and reversible increases in $[Ca^{2+}]_i$ in Fura 2-loaded MIN6 cells, with a delay in onset of ~2–3 minutes after exposure to the agonist. The same MIN6 cells showed more rapid (within 10–15 seconds) and readily reversible increases in $[Ca^{2+}]_i$ in response to ATP (100 μM) and tolbutamide (100 μM). Consistent with its effects on $[Ca^{2+}]_i$, WIN55,212-2 (1 μM) also caused a significant (P<0.001) potentiation of glucose-stimulated (20 mM) insulin secretion from MIN6 pseudoislets ((peak stimulation 478 ± 86% of 20 mM glucose-stimulated secretion, 8 minutes after application of agonist; mean secretion 287 ± 97% 20 mM glucose response over 14 minute stimulation period, n=3). In parallel experiments the CB1 receptor antagonist, AM251 (1 μM), had no detectable effect (P>0.1) on glucose-induced (20 mM) insulin secretion from MIN6 pseudoislets, (mean secretion 97.2 ± 13.8% 20 mM glucose response, n=3).
Conclusion: This study has demonstrated, for the first time, that β-cells express the DAG lipase/cannabinoid receptor signalling pathway. Pharmacological activation of CB1 receptors stimulates insulin secretion, suggesting that their activation by endogenous 2-AG generated by DAG lipase may potentiate insulin secretion, perhaps through elevation in $[Ca^{2+}]_i$.

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Dopamine D2-like receptors are expressed in pancreatic beta cells and mediate inhibition of insulin secretion
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Background and Aims: Dopamine signaling is mediated by five receptors, grouped into the D1-like (D1 and D5) and the D2-like (D2, D3 and D4) families. Effects of dopamine on pancreatic beta-cells are contradictory and documented by only limited indirect studies. Here, we investigated putative direct action of dopamine on beta-cell functions.
Materials and Methods: Expression analyses were performed by RT-PCR and immunostaining in clonal INS-1E beta-cells and isolated rat islets. Insulin secretion was measured over a 30 min glucose stimulation period and analysed by radioimmunoassay. Mitochondrial and cellular membrane potentials were monitored as fluorescence using rhodamine-123 and bisoxonol respectively, cellular calcium concentrations were measured in cells

loaded with Fura-2AM, and ATP levels were determined in cells using luciferase-mediated photon emission.
Results: We identified by RT-PCR the presence of dopamine receptors from both families (D1- and D2-like) in INS-1E cells, as well as in isolated rat islets. D2 receptor expression was confirmed at the protein level by immunostaining, revealing localization on insulin containing granules of INS-1E and primary rat beta-cells. We next examined potential effects mediated by these newly identified receptors on beta-cell function. In INS-1E cells, stimulated insulin secretion evoked by 15 mM glucose was inhibited by 10 µM dopamine (–56%, $p<0.05$) or by 5 µM of the D2-like receptor agonist quinpirole (–43%, $p<0.05$). Basal insulin release at 2.5 mM glucose was not affected by dopamine up to 100 µM. In isolated rat islets, insulin secretion stimulated by 16.7 mM glucose was inhibited by 10 µM dopamine (–49%, $p<0.001$) and by 10 µM quinpirole (–50%, $p<0.05$). Insulin release evoked by 16.7 mM glucose in FACS-purified primary rat beta-cells was also reduced by 10 µM quinpirole (–41%, $p<0.05$). Next, we measured some key parameters controlling metabolism-secretion coupling. Measurements of glucose-induced mitochondrial hyperpolarization and ATP generation in INS-1E cells showed that dopamine and D2-like agonist did not inhibit glucose metabolism. In contrast, dopamine (10 µM) reduced both cell membrane depolarization and cytosolic calcium augmentations evoked by 15 mM glucose stimulations.
Conclusion: These results show for the first time that dopamine receptors are expressed in pancreatic beta-cells and mediate direct action of dopamine in these cells. Moreover, dopamine inhibited glucose stimulated insulin secretion, an effect attributed to D2-like receptors. Regarding the molecular mechanisms implicated in dopamine-mediated inhibition of insulin release, our results point to distal steps in metabolism-secretion coupling. Thus, dopamine might play a role in glucose homeostasis in physiological and pathological states.

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Important mediators of glucose stimulated proinsulin synthesis in pancreatic β-cells
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Background and aims: Glucose acutely stimulates proinsulin (PI) synthesis in pancreatic β-cells. However, the mechanism by which this occurs is poorly understood. Therefore, the aim of this study is to understand the molecular mechanisms by which glucose stimulates PI synthesis.
Materials and methods: This study was performed in both the pancreatic β-cell line MIN6 and isolated Islets of langerhans. Cells were incubated at either low or high glucose for 1 h and the sedimentation of the preproinsulin (PPI) mRNA in polysome gradients determined. Additionally, the redistribution of the PPI mRNA between subcellular fractions was also analysed by biochemical subcellular fractionation procedures. The contribution of the redistribution of the PPI mRNA on PI synthesis was assessed by in situ and in vitro translation assays of mRNA isolated from ER membranes.
Results: In this study we demonstrate in pancreatic β-cells that glucose stimulates the recruitment of ribosome associated PPI mRNA, located in the cytoplasm, to the ER, the site of PI synthesis and that this plays an important role in glucose stimulated PI synthesis. Interestingly, glucose has a greater stimulatory effect on the recruitment of PPI mRNA to the ER than on other mRNAs encoding secretory proteins. This, as far as we are aware, is the first example whereby mRNAs encoding secretory proteins are selectively recruited to the ER and provides a novel regulatory mechanism for secretory protein synthesis. Contrary to previous reports, and importantly in understanding the mechanism by which glucose stimulates PI synthesis, we demonstrate that there is no large pool of 'free' PPI mRNA in the cytoplasm and that glucose does not increase the rate of de-novo initiation on the PPI mRNA. However, we show that glucose does stimulate the rate of ribosome recruitment onto ribosome associated PPI mRNA.
Conclusions: Our data provides evidence that the selective recruitment of PPI mRNA to the ER, together with increases in the rate of initiation are important mediators of the acute stimulation of PI synthesis in response to glucose in β-cells.
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Regulation of insulin secretion I

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In vitro characterization of nutrient-induced insulin secretion from normal human islets
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Background and Aims: In vitro studies of islets from type 2 diabetic patients will be necessary to identify the cellular mechanisms underlying the defects in insulin secretion (IS) characterizing the disease. The validity of such studies will depend on the reliability of the control responses used as a reference. Because of the difficulties in obtaining well preserved human islets, the characteristics of IS by normal human β-cells remain poorly defined: greater variability and poorer responsiveness than rodent β-cells are generally reported. This study aimed at characterizing IS from normal human islets.
Materials and Methods: The pancreas of adult organ donors (20–60 y; 22–29 BMI) was digested with liberase HI in a novel polymethylpentene chamber, and the isolated islets (5000/g pancreas) were purified (on average 75% final purity) on Ficoll gradients. After 1-3 days of culture in RPMI medium containing 5 mM glucose (G5), islets were placed in perifusion chambers (Krebs medium, collections every 2 min) for dynamic measurement of IS which was expressed as % of the islet content. Each protocol was repeated with at least 5 preparations.
Results: Increasing the glucose concentration from G1 to G15 induced biphasic IS with a prominent first phase (average peak increase of 25 ×) and a sustained, flat second phase (13 × increase). Both phases were approximately doubled when β-cell cAMP was raised by 1 µM forskolin. The central role of K-ATP channels in the response to glucose was established by its suppression with 0.1 mM diazoxide and restoration by 0.1 mM tolbutamide. Stepwise increases from G0 to G30 (30 min at each of 8 concentrations) evoked concentration-dependent IS with threshold at G3, Km at G6 and Vmax at G15 (0.07 and 0.15% of insulin content per min, without and with 1 µM forskolin, respectively). Depolarization with 0.5 mM tolbutamide or 30 mM KCl (+ diazoxide) triggered rapid IS in G1. Subsequent application of G15 further increased IS, showing that the amplifying pathway is operative. In control medium, membrane permeant analogues of pyruvate, glutamate or succinate (5 mM) increased IS in a glucose-dependent manner, while lactate and pyruvate had no effect. Whereas glutamine alone was ineffective, its combination with leucine (5 mM) induced Ca-dependent, biphasic IS. Activation of glutamate dehydrogenase by the non-metabolized BCH (with or without glutamine) evoked rapid IS, an effect that was larger in low than high G. In contrast, the IS response to a 5 mM mixture of amino acids increased with G. Palmitate slightly augmented IS in G3 and G7 when tested at the high palmitate/albumin ratio of 5, but was ineffective at the physiological ratio of 2.
Conclusion: Nutrient-induced IS from normal human islets is larger (stimulation index) than previously reported. Its characteristics are globally similar to those of IS by rodent islets, with both triggering and amplifying pathways. The pattern of the biphasic response to glucose is superimposable on that in mouse islets, but the concentration-response curve is markedly shifted to the left (even when compared to rat islets), and responses to various nutrients are modulated by glucose changes within the physiological range.

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Sterol regulatory element binding protein1 (SREBP1) is required for the potentiation by culture at high glucose concentrations of glucose-stimulated insulin secretion from mouse islets
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Background and Aims: Whilst chronically elevated glucose concentrations have been proposed to cause a progressive inhibition of glucose-stimulated insulin secretion (GSIS) in the rat, the effects of hyperglycaemia on mouse islets is less clear. We have previously proposed that up-regulation of sterol regulatory element binding protein1 (SREBP1), a transcription factor

implicated in the accumulation of lipid and "glucolipotoxicity", may be involved in mediating these effects, at least in the rat. To test this hypothesis, we have investigated here the impact of SREBP1 deletion on glucose homeostasis *in vivo* and on the response of mouse islets to chronic hyperglycaemia *in vitro*.
Materials and Methods: Intraperitoneal glucose tolerance tests (IPGTT; 2 g D-glucose/kg) were performed on overnight-fasted 4 month old mice, with glucose measurements at 0, 15, 30, 60 and 120 min. (Accu-chek glucometer, Roche). Islets were isolated by collagenase digestion and handpicking. For hyperglycaemic culture, islets were rested for 16 h at 11 mM glucose and then incubated for 96 h at 8 or 30 mM glucose. GSIS was measured after static incubation in modified Krebs Ringer medium for 30 min. by radioimmunoassay (Linco). Data were analysed by unpaired Student's t test or one-way ANOVA as appropriate, and quoted as mean ± S.E.M.
Results: No differences in body weight or fasting blood glucose concentration were observed between wild type (C57 BL/6), SREBP1(+/–) or SREBP1(–/–) animals. SREBP1(–/–) mice displayed impaired glucose tolerance with an increase of 26.4% ($p<0.05$) in the area under the curve compared with wild-type littermates. GSIS (17 vs 3 mM glucose) was identical in islets freshly isolated from wild type (6.9 ± 3.0-fold), SREBP1(+/–) (7.8 ± 1.2-fold) and SREBP1(–/–) (9.9 ± 3.7-fold; n = 3) animals. Culture for 4 days at 30 mM glucose decreased total insulin content by > 85% in each case. Whilst insulin release at 3 mM glucose was identical in SREBP1(+/+) and SREBP1(–/–) islets after hyperglycaemic culture (2–5% of total content), release at 17 mM glucose was unaffected in SREBP1(+/–) islets (15.5 ± 5.4% of total content; n = 3) but significantly ($p<0.05$) impaired in SREBP1(–/–) islets (12.2 ± 2.8%) *versus* wild-type islets (20.8 ± 2.8%).
Conclusions: (1) SREBP1 expression is not required for the normal stimulation of insulin secretion by glucose. Thus, changes in insulin sensitivity may contribute to impaired glucose tolerance in SREBP1(–/–) mice. (2) Contrary to expectations, extended culture of mouse islets at elevated glucose concentrations is shown to increase glucose-stimulated insulin secretion in the face of a substantial decrease in total islet insulin content. (3) SREBP1 is required for the potentiation of GSIS provoked by hyperglycaemia, implicating a role for β-cell lipid synthesis in this adaptation.
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A non metabolizable fatty acid analogue mimics short and long term effects of fatty acids on pancreatic insulin secretion
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Background and Aims: Fatty acids are known to have a dual effect on glucose stimulated insulin secretion (GSIS). In the short term (0–3 h) fatty acids stimulate, while in the long term (6–48 h) they inhibit GSIS. The mechanisms underlying these effects are still unclear. We used Methyl-substituted hexadecanedioic acid (MEDICA 16), a nonoxidizable fatty acid analogue to verify the non-oxidative component of the fatty acid effects.
Materials and Methods: SD rats received a single dose of MEDICA 16 or were treated with MEDICA 16 for one month. Insulin secretion was measured *in vivo*, using hyperglycemic clamp, and *in situ* using pancreas perfusion. The effect of MEDICA 16 was further studied in cultured islets.
Results: Single oral dose of MEDICA 16 resulted in doubling the area under curve (AUC) of plasma insulin and c-peptide during hyperglycemic clamp. This short term effect was also apparent *in vitro* where incubation of islets with MEDICA 16 for one hour resulted in a significant increase in GSIS (172.3 ± 22.5 *vs*. 95.7 ± 8.8 ng/ml/30 min for islets cultured with and without MEDICA 16 respectively, $P<0.05$). On the other hand, long term exposure to MEDICA 16 resulted in a significant two fold decrease in plasma insulin and c-peptide AUCs during hyperglycemic clamp. Similarly, GSIS was decreased by 1.6 fold in perfused pancreata isolated from rats treated with MEDICA 16 for one month (4963 ± 828 and 7756 ± 577 ng*min, $P<0.03$). Also, GSIS was robustly decreased in islets cultured for 48 h with MEDICA 16 (17.35 ± 2.9 and 35.2 ± 3.6 ng/ml/30 min, $P<0.004$), together with a similar two fold decrease in the responsiveness to KCl or arginine. Islet insulin content decreased by about 40% in islets incubated with MEDICA 16 for 48 h as well as in islets of rats treated with MEDICA 16 for 1 month, indicating that the decrease in insulin secretion by long term treatment with MEDICA 16 was due to islet insulin depletion. Indeed, addition of diazoxide along with MEDICA 16 to cultured islets prevented islet insulin depletion and the respective decrease in GSIS. Islet insulin mRNA content was not affected by long term MEDICA 16 treatment, indicating that MEDICA 16 did not suppress insulin transcription. Importantly, short term and long term exposure of islets to Cl-DICA, a dicarboxylic fatty acid analogue that unlike MEDICA 16 does not undergo CoA thioesterification, did not affect GSIS, indicating requirement for CoA thioesterification of MEDICA 16.
Conclusion: Similarly to fatty acids, MEDICA 16 stimulates GSIS upon short term exposure but inhibits GSIS following long term exposure, both *in vivo* and *in vitro*. The long term effect of MEDICA 16 may be accounted for by islet insulin depletion as a result of short term stimulation of GSIS not compensated by respective increase in insulin production. Lack of effect of CI-DICA implies that both, long term and short term effects of MEDICA 16 and fatty acids are mediated by the respective acyl-CoA. Our results do not support a requirement for β-oxidation for fatty acid inhibition of GSIS

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GPR119 activation increases glucose-dependent insulin secretion in insulin-producing cells and isolated rat islets
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Background and Aims: G-protein coupled receptors (GPCRs) expressed on pancreatic β-cells are known to be involved in physiological signalling systems that promote among other things glucose-dependent insulin release. Incretin hormones, such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) act in this way. Binding to their endogenous G_s-protein coupled receptors leads to an increase of intracellular cAMP via adenylate cyclase activation and thereby modulates cellular metabolism. Beside the effect on insulin secretion, it has been shown that an enhanced cAMP signal can promote cell proliferation and prevent β-cell apoptosis suggesting disease modifying potential of the mechanism. GPR119 is a recently described, β-cell specific GPCR, also targeting the cAMP signalling pathway. The aim of the study was to characterise its functionality by analysing effects of a GPR119 agonist on pancreatic β-cells and isolated rat islets.
Materials and Methods: RNAs from various human tissues were purchased and total RNAs from rat islets and cultured cell lines were isolated. The expression pattern was analysed by quantitative RT-PCR. GPR119 expression in rat pancreas sections was examined by immunohistochemistry. GPR119 agonist EX85 was used for validation of a recombinant CHO cell line expressing the human GPR119 under the control of a tetracycline-inducible promoter. Intracellular cAMP was measured with Alphascreen technology. Ins1E and isolated rat islets were used to investigate effects of EX85 on glucose-dependent insulin secretion. Insulin was measured with a rat ELISA.
Results: Expression analysis of various human tissues of GPR119 mRNA showed that the receptor is predominantly expressed in the pancreas. Further analysis of rat pancreas revealed a specific expression in the β-cells of the islets. The rat insulinoma cell lines Ins1E and Ins1 as well as the rat pancreatic ductal cell line ARIP show expression of the receptor. Induction of GPR119 expression per se stimulated cAMP synthesis in stably transfected CHO cells, suggesting constitutive activity of this receptor. EX85 turned out to be a potent GPR119 agonist in recombinant CHO-GPR119 cells increasing intracellular cAMP in a dose-dependent manner. Increase of intracellular cAMP was also observed in isolated rat islets and in the rat insulinoma cell line INS1E. As a consequence, EX 85 augmented also insulin release in these systems in a glucose-dependent manner. This effect was inhibited by the adenylate cyclase inhibitor MDL12330A.
Conclusion: These results indicate that activation of GPR119 has similar effects on β-cells as GLP-1. GPR119 is predominantly expressed in the pancreatic β-cell. Using an inducible GPR119-expressing CHO cell line, we show that the receptor is constitutively active. We also demonstrate that, despite the constitutive activity, it is possible to stimulate GPR119 with a small molecule, thereby causing an additive increase in intracellular cAMP. In isolated rat islets as well as in insulinoma cell lines, GPR119 activation results in improved response in insulin secretion in glucose-dependent manner. GPR119 agonists may therefore be useful for the treatment of diabetes, whereas the potential for mechanism related side effects may be low due to the almost β-cell specific expression pattern.

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The glucose selective insulinotropic effect of efaroxan
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Background and Aims: It is generally accepted that the block of K_{ATP} channels in pancreatic B-cells is sufficient to induce insulin secretion at a basal glucose concentration. However, some imidazoline appear not to stimulate insulin secretion under this condition even though they effectively block K_{ATP} channels in B-cells.

We compared the glucose dependence of the insulinotropic effect of efaroxan with the effect on the triggering pathway, i.e. K_{ATP} channel activity and cytosolic calcium concentration ($[Ca^{2+}]_i$), all of which were measured using mouse pancreatic islets or single B-cells.
Materials and Methods: Insulin secretion, K_{ATP}channel activity and cytosolic calcium concentration ($[Ca^{2+}]_i$), were measured using mouse pancreatic islets or single B-cells therefrom.
Results: In the presence of a non-stimulatory glucose concentration (5 mM) 100 µM efaroxan did not increase insulin secretion, but when the glucose concentration was raised to 10 mM in the continued presence of efaroxan a strong, biphasic secretion resulted, which was more than fivefold higher than that of controls. A similar, but weaker response was obtained with 30 µM efaroxan, again there was no stimulation in the presence of 5 mM glucose. 10 µM efaroxan was completely ineffective. The selectivity for stimulatory glucose concentration was lost at 500 µM efaroxan, here a marked increase in secretion was at 5 mM glucose which was further enhanced when the glucose concentration was raised to 10 mM. At 100 µM efaroxan blocked K_{ATP}channels in intact B-cells by more than 80% and produced an marked, oscillatory increase of ($[Ca^{2+}]_i$) Raising the efaroxan concentration from 100 to 500 µM produced a complete block of K_{ATP} channels and tansformed the oscillatory into a sustained increase of ($[Ca^{2+}]_i$).
Conclusion: The glucose-selective enhancement of insulin secretion by 100 µM efaroxan appears to depend on the existence of K_{ATP} channels and may be due to the oscillatory nature of the($[Ca^{2+}]_i$) increase.

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Ghrelin attenuates glucose-induced insulin release via pertussis toxin-sensitive suppression of electrical activity and Ca^{2+} signaling in rat islet β-cells

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Background and Aims: Ghrelin, isolated from the human and rat stomach, is the endogenous ligand for the growth hormone secretagogue receptor (GHS-R). We have reported that GHS-R was expressed in pancreatic islets and that ghrelin suppressed glucose-induced insulin release. In this study, we aimed to determine the intracellular mechanisms by which ghrelin suppresses insulin release in rat pancreatic β-cells.
Materials and Methods: Islets of Langerhans were isolated from Wistar rats aged 8–12 weeks by collagenase digestion. Islets were collected and used for either insulin release experiments or cyclic AMP measurements. Insulin concentrations and cyclic AMP contents were determined using ELISA kits and EIA kits, respectively. Cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_i$) in single β-cells were measured by dual-wavelength fura-2 microfluorometry. Membrane potentials and whole cell currents in β-cells were measured using nystatin-perforated patch-clamp technique.
Results: In isolated rat pancreatic islets, ghrelin (10 nM) attenuated 8.3 mM glucose-induced insulin release, while it had no effect on insulin release at 2.8 mM glucose. Ghrelin, once immunoneutralized with antiserum against active acylated-ghrelin, was no longer inhibitory on insulin release. Ghrelin also inhibited 8.3 mM glucose-induced cyclic AMP production in islets. In single β-cells, ghrelin attenuated glucose-induced first phase and oscillatory $[Ca^{2+}]_i$ increases, and these effects were blocked by a GHS-R antagonist [D-Lys^3]-GHRP-6. Ghrelin increased a tetraethylammonium-sensitive delayed outward K^+ currents in β-cells. Ghrelin also attenuated glucose-induced action potential firing by rapidly repolarizing the membrane and shortening the duration of bursting. These effects of ghrelin were not observed in the islets and β-cells following treatment with pertussis toxin (PTX).
Conclusion: This study indicates that ghrelin directly interacts with GHS-R in β-cells and thereby stimulates the PTX-sensitive pathway linked to activation of voltage-dependent delayed rectifier K^+ channels and attenuation of glucose-induced action potentials, which leads to suppression of glucose-induced Ca^{2+} signaling. Ghrelin also suppresses glucose-induced cyclic AMP production. These abilities of ghrelin to impede Ca^{2+} and cAMP signaling routes at least partly account for the inhibition of glucose-induced insulin release.

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Ghrelin inhibits glucose-stimulated insulin secretion via induction of IA-2beta

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Background and Aims: Ghrelin is a newly discovered peptide and an endogenous ligand for growth hormone secretagogue (GHS) receptor. Ghrelin has been shown to possess various central and peripheral effects including growth hormone secretion, food intake, gastric and cardiac effects. Ghrelin and the GHS receptor are expressed also in pancreatic islets. We have identified several ghrelin-induced genes by PCR-select subtraction method, among which is a β-cell autoantigen for type 1 diabetes, IA-2β. Since IA-2β locates in seceretary granules of islet cells and ghrelin has been reported to inhibit insulin secretion, we have investigated a possible link among ghrelin, IA-2β, and insulin secretion.
Materials and Methods: Male C57BL/6J mice were administrated by intraperitoneal injection of 10 µg of ghrelin or saline, sacrificed, and extracted RNA. Ghrelin-induced genes were identified by PCR-select cDNA subtraction methods, and confirmed by Northern blot. MIN6 insulinoma cells were treated by various concentartions of ghrelin for different periods and IA-2 or IA-2β mRNA was analysed by quatitative PCR or Northern blot. Insulin concentrations in media were quantified by ELISA(Linco Research). For overexpression experiments, IA-2 and IA-2β cDNAs were cloned into pcDNA3.1 and transfected to MIN6 cells. siRNA for IA-2β was transfected to MIN6 cells by RNAiFect Transfection Reagent(QIAGEN).
Results: We have identified several ghrelin-induced genes and one of them was IA-2β. Administration of ghrelin increased IA-2β mRNA in both mouse brain and pancreas in Northern analysis. Ghrelin inhibited 22.2 mmol/l glucose-stimulated insulin secretion(GSIS) in MIN6 cells dose-dependently; 11.9-fold (0nmol/l of ghrelin), 13.8-fold (0.1nmol/l), 7.4-fold (1nmol/l), 3.8-fold (10nmol/l) increase compared to 3.3 mmol/l glucose, respectively. Ghrelin also increased IA-2β but not IA-2 mRNA expression in MIN6 cells; 1.68-fold (0.1nmol/l of ghrelin), 2.34-fold (1nmol/l), 2.85-fold (10nmol/l) increase compared to 0nmol/l ghrelin. Overexpression of IA-2β but not IA-2 inhibited 22.2 mmol/l GSIS; 2.36-fold (control), 2.35-fold (IA-2), 1.39-fold (IA-2β) increase compared to 3.3 mmol/l glucose. Moreover, transfection of siRNA for IA-2β ameliorated 10nmol/l ghrelin's inhibitory effects on 22.2 mmol/l GSIS; 1.66-fold (control siRNA), 2.75-fold (IA-2β siRNA) increase compared to 3.3 mmol/l.
Conclusion: These findings strongly suggest that inhibitory effects of ghrelin on GSIS are at least partly due to increased expression of IA-2β induced by ghrelin.
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Potentiation of insulin secretion by antagonizing the urotensin II receptor. Study in the perfused rat pancreas

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Background and Aims: Urotensin II (UII), a vasoactive somatostatin-like peptide, is the endogenous ligand of the orphan G protein-coupled receptor GPR14 (UT receptor). We have previously demonstrated that, in the perfused rat pancreas, UII infusion blocks the insulin response to glucose as well as to non-glucose stimuli, and that immunoreactive UII is present in pancreatic extracts. On the other hand, circulating levels of immunoreactive UII have been reported to be elevated in type 2 diabetic patients. To investigate the influence of endogenous UII on beta cell function, we have tested the effect of a specific UII receptor antagonist, ACT-058362 (palosuran), on insulin secretion.
Materials and Methods: The study was performed in the perfused rat pancreas. Insulin was measured by RIA. Palosuran, kindly supplied by Actelion Pharmaceuticals, Switzerland, is a nonpeptidic compound shown to antagonize the specific binding of ^{125}I-UII on natural and recombinant cells carrying the human UT receptor, as well as to inhibit UII-induced contraction of rat aortic rings.
Results: Synthetic UII (10 nmol/l, Peptide Institute, Japan) blocked the insulin release elicited by an increase in perfusate glucose concentration from 5.5 to 9 mmol/l: 6 ± 3.8, SEM, ng/15 min vs. 61.7 ± 11 in controls; $p<0.05$. Coinfusion of 1 µmol/l palosuran abolished this inhibition (65 ± 16 ng/15 min). Palosuran alone significantly potentiated ($p<0.05$) the

insulin response to glucose (at 10 µmol/l, 132 ± 27 ng/15 min; at 3.14 µmol/l, 181 ± 60 ng/15 min; and at 1 µmol/l, 137.8 ± 16 ng/15 min; controls, 67 ± 14 ng/15 min). Palosuran was without effect on the insulin secretion elicited by arginine (5 mmol/l) or carbachol (1 µmol/l).
Conclusion: In the rat pancreas, palosuran, a specific UII receptor antagonist, potentiates the insulin response to glucose but not to arginine or carbachol. This suggests that endogenous UII interferes with distinct steps of the insulin secretion-coupling mechanism. The potentation of glucose-induced insulin secretion by palosuran supports the concept of UII as an insulinostatic agent and, thus, as a potential diabetogenic factor.
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Regulation of insulin secretion II

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Paradoxical inhibition of insulin release by glibenclamide, ouabain and carbamylcholine in pancreatic islets from ω3 fatty acid-depleted rats
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Background and aims: We have recently reported that, as judged from the time course for $^{86}Rb^+$ net uptake by pancreatic islets obtained from either control rats or ω3 fatty acid-depleted animals (2nd generation), the influx of K^+ by the ouabain-sensitive Na^+/ K^+-ATPase is severely impaired in the latter animals, without any significant change in the fractional K^+ outflow rate. The present study mainly aims at investigating whether such a cationic perturbation coincides with alteration of the islet secretory response to selected insulin secretagogues.
Methods: Pancreatic islets isolated by the collagenase procedure from ω3-depleted rats (2nd generation) were incubated for 90 min in a salt-balanced medium containing 8.3 mM D-glucose for measurement of insulin release. The mean values (±SEM) for insulin output mentioned below refer to 12–20 separate determinations.
Results: In the islets from ω3-depleted rats, the release of insulin induced by D-glucose (8.3 mM) was decreased ($P<0.001$) from 106 ± 11 to 18 ± 3 µU/islet in the presence of the organic calcium-antagonist verapamil (20 µM). It was increased ($P<0.005$) to 248 ± 18 and 186 ± 21 µU/islet, respectively by the tumor-promoting agent 12-O-tetradecanoylphorbol-13-acetate (TPA; 1.0 µM) and the mould metabolite cytochalasin B (21 µM). Unexpectedly, however, the output of insulin evoked by D-glucose was decreased ($P<0.001$) to 33 ± 5 µU/islet by ouabain (1.0 mM), to 51 ± 6 µU/islet by the cholinergic agent carbamylcholine (0.1 mM) and to 56 ± 7 µU/islet by the hypoglyceamic sulfonylurea glibenclamide (5 µM).
Conclusion: The present results document that, in islets from ω3-depleted rats, glucose-stimulated insulin release remains a Ca^{2+}- dependent process involving the contractile activity of the microfilamentous cell web and enhanced by TPA. At variance, however, with the situation otherwise found in islets from control rats, carbamylcholine, ouabain and glibenclamide provoked a paradoxical inhibition of glucose-stimulated insulin release in the islets from ω3-depleted rats. The latter finding suggests that the alteration of the Ca^{2+} content of such islets, as resulting, in part at least, from the impaired activity of the Na^+/K^+-ATPase and as documented in other experiments, reveals an inhibitory component of the insulinotropic action of the three secretagogues under consideration. For instance, the inhibition by ouabain of the Na^+/K^+-ATPase may provoke a further decrease in the K^+ content of the islets from ω3-depleted rats, a situation known to impair glucose-stimulated insulin release. Likewise, inhibition of insulin release by glibenclamide was previously observed in the islets from normal rats exposed to 2-ketoisocaproate (10 mM).

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The role of phosphodiesterase 3B in the regulation of first phase insulin secretion
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Background and Aims: The stimulatory effect of hormones such as glucagon-like peptide-1 (GLP-1) on β-cell insulin release is mediated through the second messenger cAMP. The level of cAMP is regulated through the balancing activities of adenylyl cyclase and cyclic nucleotide phosphodiesterases (PDE) that hydrolyze cAMP to 5'AMP. It has been shown that the PDE3B isoform is important for regulation of signals that potentiate glucose-stimulated insulin secretion through cAMP as well as for glucose-stimulated insulin secretion per se. Inhibition of cAMP-degrading phosphodiesterase (PDE) 3 has been shown to augment insulin release *in vivo* in mice and *in vitro* in human and rat islets. In the reverse situation, when overexpressing PDE3B in clonal β-cells and in rat pancreatic islets the insulin secretory capacity is reduced. More detailed studies of perfused isolated mouse islets have revealed that PDE3B is particularly important for the first phase of insulin secretion. PDE3B is a membrane associated protein but the exact membrane localization in the β-cell is not known. We hypothesize that PDE3B is localized to the exocytotic machinery and regu-

lates (a cAMP pool associated with potentiation of) insulin release. In this study we aimed to in further detail study the functional role of PDE3B in insulin secretion.
Materials and Methods: Two model systems were used in this study; (i) INS-1 (823/13) cells, subjected to adenovirus (AdPDE3B)-mediated overexpression of PDE3B and (ii) isolated islets from mice (RIP-PDE3B/7) β-cell-specifically overexpressing PDE3B.
Results: To study the involvement of PDE3B in the first phase of insulin secretion INS-1 (823/13) cells and isolated islets from RIP-PDE3B/7 mice were stimulated with known stimulators of first phase insulin secretion. INS-1 (823/13) cells infected with AdPDE3B virus to attain a 7-fold overexpression of PDE3B were stimulated with 60 mM K^+ for 5 min in the presence of 1 mM glucose. The overexpression of PDE3B reduced the insulin secretory response to 60 mM K^+ with 23% compared to control infected (Adβ-gal) cells. This effect was reversible with the addition of 10 μM of the PDE3-selective inhibitor OPC3911. Similarly, stimulation of AdPDE3B infected INS-1(832/13) cells with 20 mM L-arginine in the presence of 1 mM glucose resulted in a 25% reduction in insulin secretory response compared to control infected cells. Parallel experiments on islets isolated from RIP-PDE3B/7 mice that overexpresse PDE3B 7-fold, confirmed a 25% reduction in insulin secretion in response to 60 mM K^+ during the same conditions compared to islets from wild-type littermates. However the response to 20 mM L-arginine during 1 mM glucose conditions was not reproducible in isolated islets from RIP-PDE3B/7 mice.
Subcellular fractionation of INS-1 (823/13) cells using density gradient centrifugation (Percoll™) and subsequent immunoblot analysis showed that PDE3B is localized both to the plasma membrane and the insulin granule fraction. The localization of PDE3B was confirmed with PDE3B-activity measurements which revealed a PDE3B activity of 10–12 pmol/min/4*10^8 cells in the plasma membrane and in the insulin granule fraction respectively.
Conclusion: Our data ascribes PDE3B a functional role in the first phase of insulin secretion. The localization of PDE3B to the insulin granule fraction suggests a direct role in the exocytotic machinery in β-cells.
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Does a reduction of the intracellular GABA concentration contribute to the stimulation of islet insulin secretion by α-ketoisocaproic acid?
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Background and Aims: Several α-ketoacids (α-ketoisocaproic (KIC), and others) induce a sustained and biphasic release of insulin whereas their corresponding amino acids stimulate a transient response (L-leucine) or failed to do it (L-Ileu and L-Norleu). They are supposed to feed the Krebs-cycle by increasing the availability of α-ketoglutarate after their transamination with either endogenous, or L-glutamine-derived (exogenous), L-glutamate. We have previously demonstrated that L-glutamine-derived L-glutamate is predominantly decarboxylated to GABA in islets. Therefore, we are investigating how KIC and other homologous α-ketoacids affect the metabolism of GABA in isolated rat islets.
Materials and Methods: Islet amino acids were separated by reverse-phase HPLC after their derivatization with o-phtalaldehyde and quantified by fluorescence detection (pmol/μg islet protein). Overall rates of L-glutamine metabolism were measured as the production of $^{14}CO_2$ from L-[U-^{14}C]glutamine (pmol/islet × hour).
Results: KIC (10 mM) decreased the islet content of GABA in the absence (11.2 ± 1.1,n=5, vs.19.4 ± 1.0,n=13;p<0.001) and presence of 0.5 mmol/l L-glutamine (20.8 ± 2.0,n=6,vs. 45.3 ± 4.5,n=6;p<0.001), and this effect was not modified by 250 μM diazoxide. GABA release into the medium during 1 hour incubation was not modified by 10 mM KIC (8.9 ± 0.9, n=6 vs. 8.2 ± 0.7, n=6; N.S.) irrespective of the presence of 100 μM SKF-89976A (a specific inhibitor of neuronal GABA-uptake). No increase of the intracellular L-leucine concentration was induced by 10 mM KIC. $^{14}CO_2$-Production from 0.5 mM L-[U-^{14}C]glutamine was enhanced (+25%) by 10 mM KIC (8.4 ± 0.4,n=6,vs. 6.7 ± 0.3, n=6;p<0.01) and it was suppressed by the simultaneous presence of 10 mM allylglycine (5.7 ± 0.2, n=6,vs.8.4 ± 0.4, n=6; p<0.001). At 10 mM L-[U-^{14}C]glutamine, 10 mM KIC augmented still more (+66%) $^{14}CO_2$-production (20.8 ± 2.2, n=4, vs.12.5 ± 0.7, n=4; p<0.02) which was not affected by 10 mM allylglycine. The latter, a known GAD inhibitor, reduced islet GABA content and enhanced the secretory response to 10 mM KIC (27.7 ± 1.8,n=5,vs.18.0 ± 1.3 pg insulin/min × islet, n=6; p<0.002).
Conclusion: 1) KIC is not substantially transaminated to L-leucine. 2) It does not increase islet GABA release in long term incubations, hence it probably reduces islet γ-amino acid content favouring its metabolism in the "GABA shunt". 3) An increased "GABA-shunt" metabolism might feed additional succinic acid into the Krebs cycle and contribute to a greater ATP generation. Or, it might simply relief a hypothetical inhibition of insulin secretion induced by high levels of intracellular GABA.

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Do adenosine receptor antagonists have an antidiabetic effect mediated by A_{2B} receptors?
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Background and aims: Compounds interacting with adenosine receptors may be used as antidiabetic drugs. Aminophylline and Pentoxifyllin as non-selective adenosine receptor antagonists show an antidiabetic potential by a decrease of the hepatic glucose production and by an increase of insulin release. A_{2B} adenosine receptor antagonists have been hypothesized to be active as a new class of type 2 antidiabetics via inhibition of hepatic glucose production
Materials and methods: By using the A_1 agonist CHA (*N*6-cyclohexyl-adenosine), the A_{2A} agonist CGS-21680 (2-p-[2-Carboxy-ethyl]phenethyl-amino-5'-N-ethylcarbox-amidoadenosine), the A_3 agonist Cl-IB-MECA (2-chloro-*N*6-(3-iodobenzyl)-9-[5-(methylcarbamoyl)-β-D-ribofuranosyl]-adenine) and the newly synthesized A_{2B} antagonists PSB-1115 (1-Propyl-8-p-sulfophenylxanthine) and PSB-53 (4-(1-Butyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-yl)-benzoic acid) we tried to find out by which receptors adenosine effects on blood glucose and plasma insulin are mediated. We measured insulin release of INS-1 cells, blood glucose and plasma insulin in rats (insulin RIA, enzymatic glucose assay).
Results: *In vitro* experiments with INS-1 cells indicate that the A_1 and A_3 agonist inhibit insulin secretion where as the A_{2A} agonist was less effective. Binding assays with an A_1/A_{2B} - ([3H]PSB-298) and an A_1 radioligand ([3H]CHA) show that PSB-1115 and PSB-53 are selective for A_{2B} binding sites in INS-1 cells. *In vitro* tests with INS-1 cells show that both compounds have no effect on insulin release by themselves. They antagonize the NECA (5'-N-ethylcarboxamido-adenosine, a non-degradable adenosine analogue)-induced inhibition of insulin release, an effect that could not be explained by an A_{2B} antagonism (→ decrease of cAMP). The in vivo tests using Wistar rats show that NECA reduces plasma insulin levels and increases blood glucose. The A_1, A_{2A} and A_3 agonist also decrease plasma insulin and the A_{2A} and A_3 agonist increase blood glucose while the A_1 agonist seems to have no effect. PSB-1115 and PSB-53 reduce NECA-induced increase of blood glucose while only PSB-53 antagonizes the NECA-induced reduction of plasma insulin. The effect on blood glucose could be explained by an A_{2B} antagonism (→ decrease of cAMP). In GK rats plasma insulin levels are increased by PSB-1115.
Conclusion: NECA induced reduction of insulin secretion could be mediated by all adenosine receptor subtypes. The newly synthesized PSB-1115 and PSB-53 interact selectively with A_{2B} binding sites. The *in vitro* studies indicate an antidiabetic effect of both A_{2B} antagonists by antagonizing NECA induced inhibition of insulin release, while in the in vivo studies only PSB-53 shows an antagonism against the NECA-induced decrease of insulin. The antagonistic effect of PSB-1115 and PSB-53 on NECA induced increasing plasma glucose might be explained via A_{2B} antagonism.

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Enhanced insulin secretion from β-cells in islet-like structures is caused by anatomical configuration rather than alterations in gene expression
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Background and Aims: Disrupting islet architecture results in reduced insulin release from β-cells. We have previously demonstrated the importance of homotypic β-cell interactions by configuring insulin-secreting MIN6 cells as islet-like structures (pseudoislets), which show enhanced secretory responses to nutrients. Here we address whether cell-cell interactions influence MIN6 cell secretory responses to non-nutrient stimuli, and widen our study to include the βTC3 cell line and the glucagon-secreting αTC1 cell line. We also address the contribution to the improved secretory phenotype of changes in cellular ultrastructure, and of alterations in gene expression.
Materials and Methods: MIN6, βTC3 and αTC1 cells were maintained as monolayers, or configured as pseudoislets by culturing on a gelatin-coated

substrate. Secretory responses were measured in perifusion experiments by radioimmunoassay, and ultrastructure was assessed by transmission electron micrography. Differential gene expression was assessed in MIN6 monolayers and pseudoislets by Affymetrix gene chip analysis and by quantitative RT-PCR.
Results: Glucose-induced (20 mM) insulin secretion from MIN6 cells configured as pseudoislets (PI) was significantly greater than that from equivalent monolayer (M) cells (PI, 1030 ± 224% basal; M, 354 ± 23%, P<0.01 mean±SEM, n=5) and similar effects were observed using βTC3 cells (PI, 3867 ± 810%; M, 509 ± 35%, P<0.01, n=4). MIN6 pseudoislets also showed enhanced responses to non-nutrient stimuli such as tolbutamide (PI, 934 ± 434%; M, 322 ± 68%) and carbachol (PI, 763 ± 46%; M, 259 ± 6%). In contrast, arginine-induced (10 mM) glucagon secretion from αTC1 cells was independent of the anatomical configuration of the cells (PI, 480 ± 110% basal; M, 440 ± 80, n=4, P>0.2). Disaggregation of pseudoislets resulted in an immediate reduction of glucose-induced (20 mM) insulin secretion (PI, 2176 ± 605% basal; dispersed PI, 318 ± 74%; M, 234 ± 58%), which could be reversed by reconfiguring the cells as pseudoislets. Analysis of gene expression also suggested that configuration as pseudoislets did not cause a persistent change in the MIN6 cells, with no detectable alterations in the expression of secretion-associated genes, including cell adhesion molecules, connexins and metabolic enzymes. However, there was a significant increase in preproinsulin mRNA, consistent with increased insulin secretion (M, 102.5 ± 13.6 copies per 10^3 copies of β-actin; PI, 161.6 ± 21.9, P<0.05). The ultrastructure of MIN6 cells in pseudoislets was also consistent with increased secretory activity, with more rough endoplasmic reticulum, and insulin secretory granules localized immediately below the plasma membrane.
Conclusion: Interactions between β-cells improve insulin secretion in response to both nutrient and non-nutrient stimuli. The improved secretory responses cannot be attributed to changes in gene expression, and the effect is not observed in α-cells. These observations suggest that the underlying mechanism is an intrinsic property of β-cells which is enabled by the close cell-cell apposition in islet-like structures.
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The zinc transporter ZnT-8 enhances insulin secretion in INS-1E cells
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Background and Aims: Zinc is an important component of the cell survival and participates in several proteins structure and function. Moreover, zinc has a unique role in some specialized cells, such as pancreatic beta cells in which it is necessary to form zinc-insulin crystals in secretion vesicles. Not only zinc is necessary to insulin storage in secretory vesicles, but large amounts of zinc are locally secreted in the extracellular matrix together with insulin and play a role in islet cells paracrine/autocrine communication. Therefore, pancreatic beta cells undoubtedly need very efficient and specialized transporters. We previously identified a pancreas-specific zinc transporter, ZnT-8, which co-localized with insulin in INS-1 cells. Herein we studied its localization in human pancreas and whether this transporter was directly implied in insulin secretion.
Materials and Methods: The zinc transporter ZnT-8 was observed by confocal immunofluorescence on paraffin-embedded pancreas tissue sections with a specific antibody and revealed with a Cy3-conjugated secondary antibody. Insulin was observed with a mouse monoclonal antibody in the same tissue section and revealed with a secondary antibody conjugated with FITC. INS-1E cells were transfected and selected to stably express ZnT-8 as a fusion protein with EGFP. Parental and stable ZnT-8 expressing INS-1E cells were treated with indicated doses of glucose, with or without secretagogue, before insulin secretion determination by ELISA.
Results: In human pancreas, we observed that ZnT-8 protein was only localized in islets. Moreover, ZnT-8 was co-localized with insulin in insulin-containing beta cells of the islets, suggesting a close relationship between this transporter and insulin pathways. We next investigated the role of ZnT-8 on insulin secretion and found that overexpression of ZnT-8 in the rat beta cell model INS-1E cells significantly increases insulin secretion in a glucose-dependant manner.
Conclusion: We concluded that the recently identified zinc transporter ZnT-8 is indeed specific of human pancreatic beta cells, and that it may have an important role in the multiple regulation pathways of insulin synthesis/secretion. Therefore, it could be a suitable target for triggering insulin release from beta cells, but further work is obviously needed to understand its role at a molecular level.

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Effective and prolonged reduction of intact proinsulin and other markers of β-cell dysfunction by short term supplementary prandial insulin therapy (SIT) with insulin aspart in patients with type 2 diabetes
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Background and Aims: Supplementary insulin therapy (SIT) with short-acting insulin analogues supports meal-related β-cell function and reduces acute β-cell stress. This investigation was performed to assess the short-term effect of SIT with fixed doses of insulin aspart on β-cell function.
Materials and Methods: After informed consent was obtained, 20 patients with Type 2 diabetes with previous glimepiride monotherapy were included (5 female, 15 male, age: 61.6 ± 6.8 years, HbA1c: 7.0 ± 1.3%, disease duration: 6.7 ± 9.3 years, BMI: 31.1 ± 4.4 kg/m^2). Glimepiride therapy was continued for one week. Thereafter, the patients were randomized at day 7 (baseline) to either continue with their oral treatment (Gli) or to switch to a fixed s.c. dose of 8 U of insulin aspart (IA) before each meal for another week (day 14). Observation parameters were glucose, insulin, C-peptide, intact and total proinsulin, proinsulin split products, glucagon, Lactate; free fatty acids and adiponectin. In addition, an oral glucose tolerance test (OGTT) under therapy was performed at days 7 and 14.
Results: While significant reductions from baseline were seen in the IA group for the fasting morning values of insulin (from 13.1 ± 5.1 µU/ml to 10.6 ± 5.2 µU/ml, p<0.01), intact proinsulin (18.3 ± 11.2 pmol/l/10.3 ± 4.6 pmol/l, p<0.05), total proinsulin (43.3 ± 22.7 pmol/l/ 29.7 ± 14.5, p<0.01), and split proinsulin (24.9 ± 13.8 pmol/l/19.4 ± 10.8 pmol/l, p<0.01), no changes were seen for any of the observation parameters in the Gli group. The OGTT at endpoint showed comparable glucose excursions, because the IA dose chosen was too low to effectively lower postprandial glucose values. However, lower values for intact and total proinsulin, split proinsulin, and C-Peptide were seen after 1 h and 2 h in the IA group. No differences between the groups were seen for the other observation parameters.
Conclusion: Supporting meal-related β-cell function by preprandial insulin aspart therapy with a fixed dose of 8 U resulted in an overall and prolonged β-cell protection with an improved β-cell secretion profile in the morning and during a glucose challenge. Our results indicate that preprandial insulin aspart treatment might be a reasonable alternative to bed-time basal insulin injections for initiation of insulin therapy in patients with type 2 diabetes.
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Evaluation of effects of Lispro insulin plus NPH insulin regimen on beta cell function in new onset type 2 diabetic patients
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Background and Aims: We aimed to assess the effect of rapid-acting insulin analogue (insulin lispro) before each meal and intermediate-acting insulin (NPH) at bedtime treatment on metabolic control and beta cell reserve in newly diagnosed type 2 diabetes mellitus patients.
Materials and Methods: A total number of 20 subjects with newly diagnosed type 2 diabetic patients, 37–60 years old were eligible for the study if they had HbA1c values over 7%, C-Peptide level greater than 0.6 nmol/L, BMI between 20–35 at screening were randomly allocated into two different treatment groups.(group I: Insulin treated group, n=10; group II: Oral antidabetic (OAD) treated group, n=10). There were no significant differences between groups concerning age, body mass index, diabetes duration, HbA1c, basal and glucagon stimulated C-peptide, basal and stimulated glucagon proinsulin and serum lipid levels at the beginning of the study.All patients were followed up for 12 weeks. BMI, HbA1c, basal and glucagon stimulated C-peptide, basal and glucagon stimulated proinsulin levels, serum total cholesterol and triglycerids were measured.
Results: Group I had higher basal and glucagon stimulated C-peptide levels than group II after 12 weeks of treatment period (p =0.001, for both). Compared to baseline in group I basal and glucagon stimulated proinsulin levels decreased significantly (p =0.045, p =0.001; respectively). Basal and glucagon stimulated C-peptide levels increased significantly after 12 weeks (p =0.036, p =0.048; respectively). HbA1c reduced sicnificantly in both groups (p =0.013, p =0.005; respectively).

Conclusion: OADs and Lispro insulin plus NPH insulin regimen both provided metabolic control in newly diagnosed type 2 diabetes mellitus but Lispro insulin plus NPH insulin regimen was more effective in improving beta cell function. Our study showed that rapid-acting insulin analogue plus intermediate-acting insulin regimen offers an alternative treatment choice by improving beta cell function in newly diagnosed type 2 diabetic patients.

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Exocytosis in islet cells

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Involvement of cortical actin dynamics in regulated insulin secretion

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Background and Aims: Regulated insulin secretion has been linked in the past with remodelling of the actin cytoskeleton. We previously reported striking differences in actin cytoskeleton dynamics of two sublines (B1 and C3) derived from MIN6 cells with marked differences in their secretory properties. In this study, we analyse the distribution of β- and γ-isoactins in B1 and C3 cells, and in primary rat pancreatic beta cells. These actin isoforms display only 4 amino acid differences in their N-termini which could represent functional specialisation through interactions with specific actin binding proteins. In a parallel study, we use the B1/C3 cell model to explore a potential link between changes in actin polymerisation and the activation of the MAPK signalling cascade member ERK1/2, which has been recently implicated in the potentiation of glucose-regulated insulin secretion.
Materials and Methods: γ- and β-isoactin distribution was determined by immunofluorescence using confocal microscopy and newly-developed γ- and β-actin monoclonal antibodies. 3D reconstructions of z-stacks were performed with the Lsm510 software (Zeiss) and Metamorph (Universal Imaging). Phosphorylation of ERK1/2 +/- latrunculin B (10 μM) pre-treatment after glucose (20 mM) stimulation was analysed by Western Blot. Relative band density was determined with Scion Image for Windows.
Results: Immunofluorescence analysis of B1 cells show that γ-actin is organised as a network under the cell membrane while β-actin is localised only at the cell periphery and at cell-cell contacts. By contrast, in the secretion-defective C3 subline, the β-actin signal is increased and organised in cables. In rat primary beta cells, γ- and β-actin distribution is similar to that found in B1 cells, and the γ-actin network partially depolymerises after high glucose stimulation. Western Blot analysis of ERK1/2 phosphorylation levels at different time points after glucose stimulation show that B1 cells activate ERK1/2 more effectively than C3 cells (2.15-fold increase B1/C3, 10 min stimulation) and this activation by glucose is increased in C3 cells after actin depolymerisation by latrunculin B (7.5-fold increase +/– latrunculin B pre-treatment, 10 min stimulation). This treatment also results in restoration of glucose-stimulated insulin secretion in C3 cells. On the other hand, pre-incubation with the ERK1/2 inhibitor PD98059 (100 μM) does not have any effect on the actin cytoskeleton properties of B1 or C3 cells as observed by phalloidin staining of F-actin +/– glucose stimulation.
Conclusion: The differences in γ- vs. β-actin distribution detected in the B1 subline and in rat primary beta cells point towards specialised roles for these two actin isoforms. The change in distribution and β-actin signal detected in the C3 subline could explain its defect in actin remodelling. The short-term glucose-induced activation of ERK1/2 is clearly present in B1 cells and partially defective in C3 cells. This activation is increased after actin depolymerisation by latrunculin B in C3 cells. On the other hand, inhibition of ERK1/2 activation does not affect actin dynamics of B1 cells, indicating that the activation of ERK1/2 is a downstream effect of glucose stimulation and actin depolymerisation.

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Cyclic AMP and myosin 5a are involved in insulin granule transport during late phase insulin secretion

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Background and Aims: It has previously been demonstrated that glucose stimulates intracellular insulin granule mobility and that kinesin 1 is responsible for microtubule-mediated granule translocations. In this study we investigated the importance of cAMP and the actin-based molecular motor myosin 5a for insulin granule transport and insulin secretion.
Materials and Methods: Granule mobility in insulinoma cells was studied by confocal imaging and TIRF microscopy. Expression of myosin 5a was down-regulated using RNAinterference and the concomitant effects on

insulin secretion was investigated by hormone release measurements and capacitance recordings.
Results: Addition of the cAMP-elevating compound forskolin stimulated microtubule-mediated granule mobility by 79% ($p<0.01$) after a delay of ~10 minutes. In contrast to glucose, cAMP had a stronger effect on the frequency of translocations than on the translocation velocities. This indicates that glucose and cAMP act via different regulatory mechanisms, which both stimulate central granule mobility during late phase insulin secretion. At the cell periphery insulin granule recruitment is restricted by a dense network of actin filaments. Silencing of the actin-motor myosin 5a reduced stimulated hormone secretion by 46% ($p<0.05$) and single-cell exocytosis by 42% ($p<0.05$). Silencing of Slac2 c/MYRIP, which links insulin granules to myosin 5a, resulted in similar inhibition of single-cell exocytosis. Antibody inhibition of the myosin 5a-Slac-2 c/MYRIP interaction significantly reduced the recruitment of insulin granules for release. The pool of releasable granules independent of myosin 5a activity was estimated to ~550 granules. To directly investigate granule recruitment to the plasma membrane we applied TIRF microscopy and found that this type of transport is accelerated during late phase of insulin secretion. No such acceleration was observed after silencing of myosin 5a. This indicates that myosin 5a-mediated transport is involved in the recruitment of granules to the plasma membrane during late phase insulin secretion.
Conclusion: **(1)** Both central and peripheral insulin granule mobility is intensified during late phase insulin secretion. **(2)** cAMP and glucose stimulate central granule mobility via different mechanisms. **(3)** Peripheral actin-based granule translocations are mediated by myosin 5a.

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Direct measurement of fusion pore diameter during 'kiss-and-run': implications for the release of insulin and low molecular weight compounds

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Background and Aims: Insulin and ATP are released from beta-cells by exocytosis of large dense-core secretory vesicles (LDCVs) while gamma-aminobutyric acid (GABA) is released by the exocytosis of synaptic-like microvesicles (SLMVs). Many of these vesicles do not fully integrate with the plasma membrane but instead release their contents by a transient interaction with the plasma membrane through a semi-stable fusion pore. Thus, the fusion pore diameter will determine which vesicular components are released during these so-called „kiss-and-run" events.
Methods and Materials: Using the cell-attached capacitance technique, exocytosis of single vesicles was monitored in membrane patches of intact rat beta-cells. The size of single exocytotic vesicles and the diameter of exocytotic fusion pores were calculated based on changes in membrane patch capacitance and conductance, respectively. We specifically analysed transient „kiss-and-run" exocytotic events.
Results: Transiently fusing LDCVs had an average diameter of 368+/–29 nm, and were 1.7+/-0.8 s in duration (ranging from 70 ms and 11.4 s). Fusion pores observed during these events had an average conductance of 233+/-51 pS. This is equivalent to a pore diameter of 1.7+/–0.2 nm, which is not sufficient for passage of insulin (which is ~2.5 nm wide at its smallest axis). These pores will however permit the passage of ATP, and we calculated an ATP release time constant of 276 ms. The transient fusion of SLMVs (74+/–6 nm diameter; 0.9+/-0.2 s duration) was associated with the opening of fusion pores with an average diameter of 1.5+/–0.2 nm. Although slightly smaller than the transient LDCV fusion pore, this is sufficient for release of the relatively small GABA molecules with a time-constant of only 1.8 ms.
Conclusions: Since GABA release is predicted to occur quickly transient fusion of synaptic-like vesicles likely serves a separate purpose, such as rapid refilling of the readily releasable SLMV pool since these vesicles can be re-filled with GABA directly. The transient fusion pore present during LDCV „kiss-and-run" however acts as a molecular sieve, preventing insulin release but permitting ATP release, which would be complete during the longer fusion events.

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Reduced stimulation by cAMP in insulin-secreting cells overexpressing a truncated SNAP-25

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Background and Aims: Insulin is released from pancreatic B-cells through Ca^{2+}-dependent exocytosis.Cyclic AMP-increasing agents such as GLP-1 stimulates this process via both a PKA-dependent and a PKA-independent pathway. The latter is mediated by cAMP-GEFII, which interact with RIM. RIM has been suggested to be associated with the L-type Ca^{2+}-channel and SNAP-25. PKA have in chromaffin cells been suggested to phosphorylate SNAP-25. The aim of this study was to investigate if cAMP-stimulated exocytosis requires SNAP-25 and whether the interaction with SNAP-25 by cAMP acts through the PKA-independent or PKA-dependent pathway.
Materials and Methods: INS-1 cells were transiently co-transfected with eGFP and a pcDNA3 vector containing wild-type SNAP-25 (SNAP-25(1-206)) or a truncated SNAP-25 (SNAP-25(1–197)) or Botulinumtoxin A (BtxA) for 48 h using Effectene. Exocytosis was measured on single GFP-positive cells as changes in membrane capacitance using the standard whole-cell configuration of the patch-clamp technique. INS-1 cells overexpressing SNAP-25(1-206) was used as control.
Results: Exocytosis was elicited by voltage-clamp depolarisations from –70 mV to 0 mV with increasing duration from 5 to 850 ms. This approach makes it possible to estimate the size of the immediately releasable pool (IRP). Changes in IRP reflect effects on the PKA-independent pathway, whereas the capacitance increases evoked by the longer depolarizations (450 ms–850 ms) are related to the PKA-dependent pathway. In cells overexpressing SNAP-25(1-206) a 50 ms depolarisation evoked a capacitance increase of 7 ± 2 fF (n=6) under control conditions. This was increased to 44 ± 10 fF ($P<0.05$; n=13) in the presence of 0.1 mM cAMP. A similar 3-fold ($P<0.01$) increase could be observed when exocytosis was elicited by a 450-ms depolarization. Accordingly the size of IRP was increased from 51 ± 12 fF (n=6) in the control cells to 182 ± 18 fF ($P<0.001$; n=13) in the presence of cAMP. In cells overexpressing the SNAP-25(1–197) the estimated size of IRP, was 53 ± 8 fF (n=26) and 86 ± 20 fF (n=9) in the absence and presence of cAMP, respectively, suggesting that the capacity by which cAMP is able to increase exocytosis is reduced by ~70% ($P<0.01$; n=9–13) compared to SNAP-25(1–206). The exocytotic response in presence of cAMP at longer depolarizations (450–850) was also significantly reduced by 60% ($P<0.01$; n=9–13) in INS-1 cells overexpressing SNAP-25(1–197). The inability of cAMP to potentiate exocytosis in the cells overexpressing SNAP-25(1–197) was overcome by the addition of wild-type SNAP-25 in the pipette-solution and the size of IRP increased to 310 ± 51 fF ($P<0.001$; n=5). Similar observation was observed in INS-1 cells overexpressing BtxA where the increase in membrane capacitance evoked by a 450-ms depolarisation was 70 ± 14 fF (n=7) and 86 ± 14 fF (n=30) in the absence and presence of cAMP.
Conclusion: 1) Potentiation by cAMP on exocytosis requires the presence of the full-length SNAP-25. 2) The reduced size of IRP in the INS-1 cells overexpressing SNAP-25(1–197) suggests an interaction between cAMP-GEFII-dependent pathway and SNAP-25. 3) cAMP might also interact with SNAP-25 through PKA-dependent phosphorylation of the protein since PKA-dependent stimulation was also strongly reduced in the INS-1 cells overexpressing the truncated SNAP-25.

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Redox control of exocytosis: regulatory role of NADPH, thioredoxin and glutaredoxin

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Background: Cellular redox state is an important metabolic variable, influencing many aspects of cell function like growth, apoptosis and reductive biosynthesis. In this study we identify NADPH as a candidate signaling molecule for exocytosis in neuroendocrine cells.
Methods: NADPH and $NADP^+$ were measured in primary beta cells and in MIN6 cells via enzymatic cycling and insulin release via radioimmunoassay. Exocytosis was measured as increases in cell capacitance. Expression of a glutaredoxin (GRX) and thioredoxin (TRX) was analyzed at the mRNA level (PCR, microarray) and protein level (Western blots, immunocytochemistry).

Results: Glucose acutely raised the [NADPH]/[$NADP^+$] ratio both in pancreatic beta cells and in MIN6 cells, and stimulated insulin release in parallel. Furthermore, intracellular addition of NADPH directly stimulated exocytosis of insulin granules. Effects of NADPH on exocytosis are proposed to be mediated by the redox proteins GRX and TRX on basis of the following evidence: (a) expression of GRX mRNA is very high in beta cells as compared to other studied tissues and GRX protein is high in islets and in brain; (b) GRX and TRX are localized in distinct microdomains in the cytosol of beta cells; (c) microinjection of recombinant GRX potentiated effects of NADPH on exocytosis, whereas TRX antagonized the NADPH effect.
Conclusion: We propose that the NADPH/GRX/TRX redox regulation mediates a novel signaling pathway of nutrient-induced insulin secretion.

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Cytoskeleton organisation may underlie decreased basal insulin secretion from cells in close physical contact
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Background and Aims: Cell-cell interaction is essential for proper function of beta cells within the islet. Beta cells are coupled through gap junctions and cell adhesion molecules that are linked to the cytoskeleton. The aim of this study was to understand better the changes that occur when beta cells are in contact with one another and how these changes account for altered insulin secretion.
Materials and Methods: An equal number of MIN6B1 cells were attached either in the centre ("confluent") or all over the surface ("dispersed") of 24-well plates with 0.5 ml medium. After 48 h in culture, cells were preincubated for 2 h or 6 h in KRB-buffer at 2.8 mM glucose and then incubated for 1 h each at 2.8 mM (basal) and 16.7 mM (stimulated) glucose. Insulin was measured by radioimmunoassay. Proinsulin trafficking and conversion were measured by pulse-chase and HPLC. Gene expression was quantified using Affymetrix MOE430A microarrays and data analysed by GeneSpring (Silicon Genetics) with differences >1.4-fold, $p<0.05$ considered significant. Results are mean±SEM, n=3 independent experiments.
Results: When cells were plated densely with extensive cell-cell interaction (confluent), they were well spread. Basal secretion after a 2 h preincubation was low (0.4 ± 0.05% content/h) and was stimulated 27-fold with 16.7 mM glucose. Dispersed cells without cell-cell interaction although attached to the culture surface remained round and did not spread. Dispersed cells showed weak glucose-stimulation of insulin secretion (2-fold) that was due to greatly elevated basal secretion (4.2 ± 0.28% content/h) rather than any difference in absolute amounts of insulin secreted vs. confluent cells at high glucose. However, if the preincubation period under basal conditions was extended to 6 h, subsequent basal release from dispersed cells decreased to 0.6% content/h and glucose was then able to elicit a 6-fold increase in secretion. No difference in insulin gene expression (0.92 ± 0.2-fold dispersed vs. confluent) could be detected by RT-PCR. HPLC analysis of radiolabelled cells showed no significant difference in proinsulin conversion in dispersed vs. confluent cells (proinsulin as percent total proinsulin + insulin synthesised after 4 h label: 5.7 ± 1.0 in dispersed cells vs. 7.4 ± 1.6 in confluent cells). Constitutive secretion of radiolabelled proinsulin was <1% total cell content/90 min for both dispersed and confluent cells. Differential gene expression in these two settings was evaluated by oligonucleotide microarrays: there were 24 identified genes over-expressed in dispersed cells and 54 in confluent cells with no differences exceeding 2.2-fold. The majority of these genes were transcription factors or involved in intracellular signalling with 5 genes over-expressed in confluent cells potentially involved in cytoskeleton organisation.
Conclusion: High rates of basal secretion underlie the poor glucose response of dispersed cells. This is not due to diversion of proinsulin from the regulated to the constitutive secretory pathway and becomes less manifest after prolonged preincubation at low glucose. We postulate that such elevated basal secretion in dispersed cells results from poor organization of the cytoskeleton in round dispersed vs. spread confluent cells due primarily to disruption of signalling from (disengaged) adhesion molecules such as E-cadherin as well as alterations in gene expression.

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A role of granular pH in insulin secretion?
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Background and Aims: Insulin granules possess a v-type H-ATPase that maintains a low intravesicular pH (pHv) by pumping protons into the lumen, while simultaneous Cl uptake maintains electroneutrality. This low pHv is important for proinsulin conversion and storage of Zn-insulin hexamers. A role in insulin secretion (IS) is also possible. Thus, it has been suggested that glucose (G) and sulfonylureas could increase release competence of insulin granules by causing further acidification. However, this hypothesis is not uncontested and is based on experiments using permeabilized cells or indirect measurements of IS (whole-cell capacitance recordings). In this study we determined the effects of G on pHv in intact islets and evaluated their significance for IS.
Materials and Methods: Intact mouse islets and clusters of islet cells were cultured for 1–2 days in RPMI medium containing 10 mmol/l G (G10). To measure pHv we used Lysosensor DND160, a fluorescent dye that accumulates in acidic compartments and permits ratiometric (ex 340–380/em 535 nm) determination of pH <6. Cytosolic pH and Ca concentration ([Ca]c) were measured after islet loading with BCECF and Fura-PE3, respectively. Cell metabolism was monitored by the NADPH autofluorescence. IS was measured with perifused islets.
Results: To identify the cellular compartments labelled by Lysosensor we measured fluorescence intensity (ex 365/em >400 nm) in loaded clusters. Beta cells exhibited a punctated fluorescence that was decreased by inhibitors of the v-H-ATPase (bafilomycin and concanamycin), by amines (NH_4Cl) that alkalinise secretory vesicles, and by degranulation after strong stimulation of IS. In vitro ratiometric calibration showed that Lysosensor detected pH changes between 4.3 and 5.5 independently of dye concentration. The probe thus seemed suitable to measure granular pH (lysosomes being only few) in whole islets, and yielded a value of 5.0 in the presence of G1. Stimulating with G15 reversibly acidified insulin granules (while increasing cytosolic pH). The effect required G metabolism (suppressed by poisons and not mimicked by non metabolized sugars), was inhibited by Cl deprivation, and was independent of [Ca]c changes (neither prevented by diazoxide nor mimicked by tolbutamide). The contribution of v-H-ATPases in the effect of G could not be established because the fluorescence was too faint after islet treatment with the inhibitors (pHv >6). Bafilomycin and amines altered metabolism and [Ca]c, and were not further used. Islet pretreatment with concanamycin (100 nmol/l for 2 h) slightly raised basal [Ca]c but did not prevent G15 from inducing typical changes with an initial lowering, a large first phase increase and oscillations in the steady state. In contrast, both phases of IS were strongly depressed under these conditions. The G15-induced rise in NADPH was unaffected but the increase in cytosolic pH was almost suppressed. Concanamycin did not impair the [Ca]c rise induced by 30 mmol/l KCl + diazoxide but inhibited (70%) IS without suppressing the amplification by glucose (larger IS at G15 than G1).
Conclusion: High glucose acutely acidifies insulin granules by a Ca-independent, metabolism-dependent, mechanism. Alkalinization of the granules by blockade of the v-H-ATPase inhibits IS at a step distal to the rise in [Ca]c. The data support the hypothesis that an acidic luminal pH is important for exocytosis of insulin granules, but cannot establish whether the additional effect of glucose plays a significant role.

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Ca^{2+} handling and insulin exocytosis regulated by hGH in insulin-secreting cells
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Background and Aims: Growth hormone (GH) is an important factor in metabolism and proliferation by pancreatic β-cells, where an increase in cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) plays an important role. Our previous studies showed that GH-stimulated a rise in $[Ca^{2+}]_i$ associated with proliferation in the β-cell (Sjöholm *et al*, J Biol Chem, 2000, 275:21033–40) and GH-induced rise in $[Ca^{2+}]_i$ in the β-cell was facilitated by Ca^{2+}-induced Ca^{2+} release through tyrosine phosphorylation of ryanodine receptors (Zhang *et al*, Mol Endocrinol, 2004, 18:1658–69).
Material and Method: In the present study, we applied hGH, bovine GH (bGH) and ovine prolactin (oPRL) to investigate the receptors and tyrosine kinases involved in GH-induced rise in $[Ca^{2+}]_i$ and insulin exocytosis in the insulin-secreting cell line BRIN-BD11.

Results: Stimulation of the cells with hGH caused tyrosine phosphorylation of JAK2 and c-SrC, which was associated with an increased $[Ca^{2+}]_i$ and insulin secretion. The hGH-induced phosphorylation of JAK2 and c-SrC was inhibited by the specific JAK2 inhibitor tyrphostin AG490 or the specific inhibitor for SrC kinases PP2. Accordingly, the hGH-stimulated rises in $[Ca^{2+}]_i$ and insulin secretion were completely abolished by AG490, while the inhibitor had no effect on insulin secretion stimulated by K^+. However, K^+-induced rise in $[Ca^{2+}]_i$ was also partially inhibited by AG490. This effect disappeared when the intracellular Ca^{2+} pools were exhausted. PP2, but not its inactive analogue PP3, partially inhibited $[Ca^{2+}]_i$, while it completely abolished insulin secretion stimulated by hGH. In contrast, K^+-induced rise in $[Ca^{2+}]_i$ and insulin secretion was not affected by the inhibitor. To investigate the receptor through which the effect of hGH was mediated, we applied bGH and oPRL in the study. Stimulation of the cells with oPRL evoked a rise in $[Ca^{2+}]_i$ and insulin secretion to a similar extent as hGH. The effects of oPRL on both $[Ca^{2+}]_i$ and insulin secretion were inhibited by AG490 or PP2. In contrast, application of bGH had no effect on insulin secretion, in spite of an increased $[Ca^{2+}]_i$ elicited by the hormone.
Conclusion: Our study suggests that hGH-stimulated rises in $[Ca^{2+}]_i$ and insulin secretion involve activation of both JAK2 and SrC kinases, and are mediated mainly through the PRL receptor in rat insulin secreting cells.

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Mechanisms behind cAMP-dependent stimulation of glucagon-secretion in A-cells
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Background and Aims: Glucagon is released from the pancreatic A-cells through Ca^{2+}-dependent exocytosis. GLP-1, glucagon and forskolin have been shown to stimulate exocytosis in the A-cell. The aim of this study was to investigate the mechanisms behind cAMP-stimulated exocytosis in mouse pancreatic A-cells.
Materials and Methods: Single mouse A-cell exocytosis was monitored as increases in cell capacitance using the perforated or standard whole-cell configuration of the patch-clamp technique. Exocytosis was elicited by application of voltage-clamp depolarization of indicated durations from –70 mV to membrane potentials between –30 mV to 20 mV to investigate the voltage-dependence of exocytosis
Results: Several mechanisms are involved in Ca^{2+}-dependent exocytosis in A-cells. These include Ca^{2+} influx through voltage-dependent Ca^{2+} channels. By applying isradipine (2 μM) and ω-conotoxin GVIA (1 μM) to single A-cells, we could demonstrate the existence of both L-type and N-type Ca^{2+} channel. Further, the exocytotic response under control conditions was inhibited by ω-conotoxin GVIA ($P<0.01$; n=4–24), whereas the increase in membrane capacitance in the presence of 0.1 mM cAMP was inhibited by the L-type channel blocker ($P<0.001$; n=7–38), suggesting that cAMP-stimulated exocytosis mainly act through L-type Ca^{2+}channels.
Next the voltage-dependence of exocytosis under control condition and 4 min after application of either glucagon or forskolin was investigated. Maximal exocytotic response under control conditions was observed when the membrane potential was depolarized to 0 mV and amounted 19 ± 3 fF (n=5). Addition of 10 nM glucagon was associated with a 3-fold (n=5;$P<0.05$) increase in the exocytotic response but did not shift the voltage-dependence of exocytosis. In the presence of 10 μM forskolin an increase in membrane capacitance was observed at a voltage as negative as –20 mV. The maximum capacitive response was increased 6-fold ($P<0.001$;n=5) compared with control. Importantly, stimulation with forskolin was associated with a 10-mV shift of the voltage dependence of exocytosis to more negative values. These effects on exocytosis did not correlate with any changes in the Ca^{2+} current amplitude. The observed shift in the presence of forskolin but not glucagon might due to the fact that the forskolin stimulation involves both PKA-independent (through cAMP-GEFII) and PKA-dependent pathways, whereas glucagon mainly acts through PKA-dependent stimulation. The specific cAMP-GEFII-agonist 8CPT-2Me-cAMP strongly stimulated exocytosis 10-fold ($P<0.001$; n=7–8) compared to control and preliminary results indicate that this stimulation can be inhibited by the L-type Ca^{2+}-channel blocker isradipine.
Conclusion: 1) cAMP-stimulated exocytosis in mouse pancreatic A-cells is associated with influx of Ca^{2+} through L-type Ca^{2+}channels. 2) The shift in voltage-dependence of exocytosis to more negative potentials by forskolin, but not by glucagon might explain why less potent cAMP-increasing agents do not stimulate glucagon-secretion from the whole islet.

PS 28
Ion channel function in islet cells

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A novel ryanodine receptor expressed in pancreatic islets and its possible role in the cyclic ADP-ribose-induced intracellular calcium mobilization
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Background and Aims: Increases in intracellular Ca^{2+} concentration are prerequisite for glucose-induced insulin secretion. Mobilization of Ca^{2+} from intracellular stores in the endoplasmic reticulum as well as Ca^{2+} influx from extracellular source is important in this process. We have proposed that the intracellular Ca^{2+} mobilization is mediated by the CD38-cyclic ADP-ribose (cADPR) system in normal islets, that is, cADPR generated by CD38 in response to glucose stimulation acts as a second messenger for Ca^{2+} release from the endoplasmic reticulum for insulin secretion. The ryanodine receptor (RyR) has been postulated to be an intracellular Ca^{2+} release channel for cADPR in pancreatic islets.
Materials and Methods: A rat islet cDNA library was screened by plaque hybridization using *RyR* cDNA fragments for *RyR1*, *RyR2*, and *RyR3*. Expression of a novel *RyR* was analyzed by RT-PCR. Expression vectors for RyRs were transfected into HEK293 cells, which then expressed them transiently. The ryanodine binding was analyzed using the HEK293 whole cell extracts. Fura 2 was loaded on HEK293 cells and Ca^{2+} mobilization was induced by caffeine with or without cADPR.
Results: We isolated a novel *RyR* cDNA from a rat cDNA library. The deduced protein of the cDNA was 4,947 amino acids with a molecular weight of 562,291. The amino acid sequence of the rat islet RyR showed 97% identity with that of human RyR2. However, the two regions were not found in the rat islet RyR. By RT-PCR with RNA from rat cardiac muscle, we found that the regions occur in the rat cardiac *RyR2* mRNA. RT-PCR with RNAs from cardiac muscle and islets of human and mouse revealed that the cardiac *RyR2* mRNA but not the islet *RyR2* has the regions. Genomic analyses of *RyR2* revealed that the islet *RyR* is generated by alternative splicing from the authentic *RyR2*. When the expression vectors for the islet-type and the authentic RyRs were transfected into HEK293 cells, the islet-type RyR2 as well as the authentic one showed high affinity [^{3}H]ryanodine binding. Intracellular Ca^{2+} release in the islet-type RyR2-transfected cells was enhanced in the presence of cADPR but not in the authentic RyR2-transfected cells. The islet-type *RyR2* mRNA was expressed in a variety of tissues such as in pancreatic islets, cerebrum, and cerebellum, whereas the authentic *RyR2* mRNA was predominantly expressed in heart and aorta. Interestingly, the authentic *RyR2* mRNA was expressed in *ob/ob* mouse islets, which was less sensitive to cADPR, but not in control C57/BL mouse islets.
Conclusion: We found a novel *RyR* cDNA in islets, which is generated from the *RyR2* gene by alternative splicing. The Ca^{2+} release from the islet-type RyR was much enhanced by cADPR, indicating the new RyR2 is an intracellular target for cADPR signaling.
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ATP-sensitive potassium channel independent pathway is involved in action mechanism of meglitinides, but not sulfonylureas
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Background and aims: Meglitinides (Nateglinide and mitiglinide), are characterized as rapid-onset and short-acting insulinotropic agents. These compounds do not have a sulfonylurea structure, but it has been postulated that insulin secretion is preceded by their binding to a Kir6.2/SUR1 complex, an ATP-sensitive potassium channel (K_{ATP} channel), and a mechanism of insulin secretory effect has been accounted for by this pathway. The aim of this study is to elucidate the action mechanism of meglitinide on insulin secretion profile more precisely including an involvement of K_{ATP} channel independent pathway, and to clarify the difference from that of sulfonylureas. The MIN6 cells used in this study had been confirmed previously to demonstrate the same manner for insulin secretion induced by glucose or insulinotropic agents as shown in isolated pancreatic islet.

Materials and methods: The MIN6 cells were incubated in the KRB buffer containing 3 mM glucose for 30 min at 37 °C. Then, the reaction buffer containing 3 µM tolbutamide, 1 nM glibenclamide, 3 µM nateglinide, or 30 nM mitiglinide was added and exchanged every 30 sec. In order to assess the effect of extracellular calcium influx and intracellular calcium release from endoplasmic reticulum on insulin secretion, 10 µM diazoxide (K_{ATP} channel opener), 3 µM verapamil (L-type calcium channel blocker) and 1 µM dantrolene (nonselective ryanodine receptor blocker), and each of agents was directly added into the incubation buffer 2 min prior to the each stimulations. Immunoreactive insulin secreted from the cells into the incubation buffer was measured by ELISA.
Results: Tolbutamide initiated insulin secretion at 60 sec of stimulation with a peak at 120 sec. Insulin secretion induced by glibenclamide observed at 150 sec and its effect lasted. Nateglinide and mitiglinide initiated insulin secretion at 30 sec with a peak at 90 sec. Addition of diazoxide or verapamil completely suppressed insulin secretion induced by tolbutamide or glibenclamide. In the same condition, however, nateglinide-induced insulin secretion was partly suppressed, and a component initiated at 90 sec of stimulation with a peak at 150 sec was still remained. This nateglinide specific component of insulin secretion was observed even in Ca^{2+} depleted medium, where tolbutamide-induced insulin secretion was confirmed to show the same profile as in the existence of verapamil. Interestingly nateglinide specific component was suppressed by dantrolene. Nateglinide-induced insulin secretion was completely suppressed by concomitant addition of verapamil and dantrolene. The release of Ca^{2+} from endoplasmic reticulum was induced by 10 mM caffeine and its action was suppressed by dantrolene. In addition, 5 mM caffeine, which enhances ryanodine receptor sensitivity, increased nateglinide specific component of insulin secretion.
Conclusion: The present results strongly suggested that the action mechanism of meglitinides on insulin secretion is not always the same as that of sulfonylureas, and the intracellular calcium release from endoplasmic reticulum, which is independent of K_{ATP} channel-dependent pathway, may play an important role on meglitinide-induced insulin secretion from pancreatic beta cells.

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L-type voltage gated calcium channels activation of extracellular regulated kinase in pancreatic β-cells

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Background and Aims: Glucose, GLP1 or an increase in extracellular K^+ can all lead to the activation of Erk through a mechanism which requires the influx of calcium through L-type voltage gated calcium channels (VGCC). However, the mechanism by which the L-type channels couple to the Erk signalling pathway is poorly understood. Therefore, the primary aim of this study is to determine the signalling pathways emanating from the L-type VGCC to Erk.
Materials and Methods: MIN6 cells and Islets of Langerhans were treated with agents that induce Erk activation through the entry of Ca^{++} through L-type VGCCs. The role of defined signalling molecules in the activation of Erk was assessed using either RNAi or dominant negative/constitutive active forms of these proteins. The activation of Erk was assessed by Western blot analysis using phospho-specific antibodies to Erk.
Results: In islets, we demonstrate that L-type VGCC mediated activation of Erk is dependent on Mek, independent of Ras, but is likely to be dependent of Rap. Using siRNA, the role of A, B and C Raf in L-type VGCC mediated Erk activation was also investigated. Preliminary results indicate that the over-expression of dominant-negative forms of calcium/calmodulin dependent protein kinase II and IV interfere with L-type VGCC activation of Erk. Additionally, we demonstrate that L-type VGCC activation of Erk is not dependant on the release of calcium from the ER.
Conclusions: L-type VGCC stimulated phosphorylation of Erk likely requires the activation of CaM kinase, mediated specifically via an increase in intracellular Ca^{++} through L-type VGCC and not through the release of Ca^{++} from intracellular stores. Additionally, GLP1 mediated phosphorylation of Erk is dependent on Mek but independent of Ras. We are currently in the process of identifying the kinase responsible for the phosphorylation of Mek.
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Morphological localization of the sulphonylurea receptor 1 in human and rodent endocrine pancreas

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Background and Aims: In pancreatic β -cells, ATP-sensitive K^+ (K_{ATP}) channels are composed of 4 inwardly rectifying K^+ channel (Kir6.2) and 4 sulphonylurea receptor 1 (SUR1) subunits. They regulate insulin secretion by controlling the membrane potential and, thereby, Ca^{2+} influx, during stimulation by nutrients and hypoglycaemic sulphonylureas. Recent studies on rat and mouse islets have suggested that K_{ATP} channels might also be localized on intracellular organelles including membranes of insulin-containing granules, mitochondria and nucleus. A role of K_{ATP} channels in the control of glucagon and somatostatin secretion is also emerging. In this study, morphological methods were used to identify the cell types containing SUR1 in rodent and human islets, and to determine the ultrastructural localisation of SUR1.
Materials and Methods: The localization of SUR1 peptide in the pancreas from wild-type and $SUR1^{-/-}$ mice (provided by J. Bryan), rats and normal human subjects was investigated by conventional (peroxidase system) and electron (gold labelling) microscopy, using an antibody directed against the nucleotide-binding domain 1 (provided by S.J. Ashcroft). SUR1 and insulin immunostaining was quantified by densitometry in islets from control and glibenclamide-treated (5 ip injections) rats. Sulfonylurea binding sites were also identified (Zeiss Apo Tome) after treatment of mouse islet cell clusters with green fluorescent glibenclamide BODIPY-FL.
Results: In the human, mouse and rat pancreas, all endocrine cells of the islets were immunolabelled for SUR1, whereas acinar and duct cells, and vascular tissues (containing SUR2) were negative. No SUR1 labelling was observed in islets from $SUR1^{-/-}$ mice. Compared to β-cells, non β-cells were labelled strongly in the mouse, weakly in the rat and similarly in humans. In β-cells of all species, the plasma membrane was distinctly stained, but SUR1 was also consistently and prominently present in the cytoplasm. Within all islet cells, glibenclamide BODIPY-FL binding appeared as numerous punctate structures, suggesting endocrine granule labelling. This was confirmed by electron microscopy showing that SUR1 was immunolocalized in insulin, somatostatin and glucagon granules. The nuclear membrane was variably labelled and mitochondria were consistently negative. In β-cells from glibenclamide treated-rats, the insulin content and SUR1 staining were similarly decreased by about 50%, whereas no change in SUR1 staining was detectable in the non β -cells.
Conclusion: This study provides the first morphological demonstration of SUR1 peptide in the plasma membrane of β-cells. It also shows that secretory granules are a major site of SUR1 protein localisation in all endocrine cell types of rodent and human islets. Whether the granules serve to translocate K_{ATP} channels to the plasma membrane or whether SUR1 has a functional role at the granule level remains to be established.
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Pancreatic islets morphology in SUR1 deficient mice

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Background and Aims: ATP-sensitive K^+ (K_{ATP}) channels, composed of sulfonylurea receptor 1 (SUR1) and pore forming Kir6.2, play a key role in the control of insulin secretion. In human congenital hyperinsulinism, the loss of this channel activity in β cells induces a continuous membrane depolarisation resulting in permanent insulin secretion and severe hypoglycemia. However, unexpectedly, K_{ATP} deficient mice, genetically modified to disrupt SUR1 gene, are normoglycemic. This study was designed to investigate whether SUR1 gene inactivation has an impact on pancreatic islets morphology that could explain the maintenance of a normal blood glucose concentration in these mice.
Materials and Methods: Pancreases were collected from young (5.7 ± 1.4 days, n=20), adult (6.6 ± 1.4 months, n=10) and old (13.2 ± 0.5 months, n=10) Wild type (WT) and SUR1 knockout (KO) mice (provided by J. Bryan). In conventional microscopy, the intra-insular distribution of α and δ cells, immunostained for glucagon and somatostatin respectively, was analysed by an original quantitative topographical approach dividing each islet surface in three equal and concentric areas and determining the percentage of α and δ cells located in each of them. The relative proportions of β, α and δ cells in each islet of all groups were calculated after a double immunostaining and the β and α cell size were evaluated by planimetry. In

electron microscopy, the nuclear area of endocrine cells, the number of mitochondria and the size of rough endoplasmic reticulum (RER) were measured.
Results: In WT young mice, α cells were equally dispersed in the central, intermediate and peripheral islet areas. With ageing, α cells were mainly distributed in a peripheral rim both in adult and old mice islets (8 ± 8% in the centre, 32 ± 15% in the intermediate, and 60 ± 20% in the peripheral areas of the old WT islets). In young mice, the SUR1KO islets morphology was not different from that of WT mice. But, in adult mice, the α cells were found also in the central parts of SUR1KO islets. This abnormal α cell distribution was strongly marked in SUR1KO old mice islets (24 ± 11%, 40 ± 9% and 36 ± 13% respectively in the central, intermediate and peripheral islet areas). On the contrary, the δ cell intra-insular distribution was similar in the WT and SUR1KO mice, whatever their age. In young SUR1 KO mice, β cell number was increased by 12% ($p<0.05$) and that of α cells was decreased by 14% ($p<0.05$), in comparison with the WT. In adult, no significant differences were observed, whereas in old SUR1KO islets, only α cell number was significantly increased (27%, $p<0.02$). The size of β and α cells increased significantly and similarly with age in both WT and SUR1KO mice. In electron microscopy, the β, α and δ cell RER was more abundant (3 times larger) in adult and old SUR1KO mice than in age-matched WT. The size of endocrine cell nuclei and the number of mitochondria were similar in both groups.
Conclusion: The adult and old SUR1KO mice islets differ from those of the WT by a change in the intra-islet α cell distribution, an increase in α cell number, and an abundant RER in all endocrine cell types. These modifications and especially the α cell location between the β cells rather than at their periphery might reflect an adaptative mechanism by which the α cells could influence β cell function and ensure normoglycemia.
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Ca^{2+}-induced Ca^{2+} release activates PLC and triggers store-operated Ca^{2+} influx in mouse β-cells
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Background and Aims: The hormones glucagon and glucagon-like peptide-1, strongly amplify nutrient-stimulated insulin secretion by raising cAMP. Among several mechanisms of action cAMP promotes Ca^{2+}-induced Ca^{2+} release (CICR) from the endoplasmic reticulum (ER) by sensitizing inositol-1,4,5-trisphosphate (IP_3) receptors. The resulting Ca^{2+} spikes trigger exocytosis of insulin granules. Similar spikes are induced by receptor-mediated activation of the IP_3-generating enzyme phospholipase C (PLC). Since the PLC activity depends also on Ca^{2+} we investigated whether CICR can stimulate the enzyme independent of receptor agonist. Release of Ca^{2+} from the ER is known to activate a store-operated cation influx into the β-cell. Although important functions have been attributed to this mechanism little is known about its detailed regulation. We therefore studied whether CICR is associated with activation of the store-operated mechanism.
Materials and Methods: Pancreatic β-cells isolated from mice were exposed to 20 mM glucose to maintain Ca^{2+} loading of the ER and shut off influx through store-operated pathway. Voltage-dependent Ca^{2+} entry was prevented by the channel blocker methoxyverapamil and by hyperpolarizing the cells with diazoxide. CICR was evoked by exposing the cells to agents elevating cAMP. Simultaneous recording of PLC activity and $[Ca^{2+}]_i$ was performed by evanescent wave microscopy on mouse β-cells transfected with the phosphatidylinositol-4,5-bisphosphate-binding pleckstrin homology domain from PLC-δ_1 fused to yellow fluorescent protein (PH-YFP) and loaded with the Ca^{2+} indicator fura red. Activation of the store-operated pathway was estimated from the rate of increase in $[Mn^{2+}]_i$ due to influx of the ion with subsequent quenching of the fluorescence from the indicator fura-2.
Results: In the presence of diazoxide and methoxyverapamil $[Ca^{2+}]_i$ remained at basal levels in glucose-stimulated mouse β-cells. Elevation of cAMP by exposure to glucagon, forskolin and IBMX induced irregular pronounced $[Ca^{2+}]_i$ spiking by CICR. These spikes were associated with temporary activation of Mn^{2+} influx, which was blocked by the store-operated channel inhibitor SKF96365. The $[Ca^{2+}]_i$ spikes were also followed by rapid and transient loss of plasma membrane associated PH-YFP fluorescence, reflecting activation of PLC. The results show that Ca^{2+} mobilized from intracellular stores by CICR is sufficient to activate PLC. Moreover, single CICR events readily activate the store-operated pathway.
Conclusion: CICR in normal pancreatic β-cells has been primarily attributed to activation of IP_3 receptors. The present data indicate that these receptors are also involved in a positive feedback loop with Ca^{2+}-activated generation of IP_3 by PLC. Such feed-back may be required for CICR to reach sufficient levels of $[Ca^{2+}]_i$ to trigger exocytosis. The fact that individual CICR events activate store-operated cation influx is consistent with ideas that this depolarizing pathway may be involved in generating the characteristic electrophysiological burst pattern in β-cells.

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Expression of sulfonylurea receptor SUR1 enhances induction of apoptosis by resveratrol but not by etoposide
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Background and aims: Sulfonylurea receptor 1 (SUR1) is the important regulatory subunit of the pancreatic ATP-sensitive K^+ channel (K_{ATP} channel) which is essential for triggering insulin secretion in the β-cell. SURs are members of the large family of ATP-binding cassette (ABC) proteins. The naturally occurring phenolic compound resveratrol is known to be a ligand of several ABC-proteins and shows structural similarity to DIDS (4,4-diisothiocyanato-stilbene-2,2-disulphonicacid), a synthetic K_{ATP} channel modulator that binds to SUR. Apart from other physiological effects, *trans*-resveratrol induces apoptosis in some cell types. In the present study, we tested wether expression of different SUR isoforms renders cells more susceptible to induction of apoptosis by resveratrol.
Materials and methods: HEK293 cells stably or transiently expressing SUR1, SUR1(M1289T) or SUR2B or sham-transfected with expression vector pcDNA3.1 were treated with resveratrol or etoposide, a classical inducer of apoptosis widely used in chemotherapy. After 24 hours, cell detachment and viability were determined using a CASY Cell Analyser System. DNA condensation and fragmentation were assessed after Hoechst 33258 staining, and caspase 3-like activity was determined by cleavage of fluorescent DEVD-substrates.
Results: Resveratrol treatment (100 μmol/l, 24 hours) resulted in intensive detachment of cells expressing SUR1 while control cells (pcDNA-cells) and SUR2B-expressing cells were less affected. In the presence of resveratrol, the number of detached SUR1-cells was about two-fold higher compared to control cells and even three times higher than that of SUR2B-cells (SUR1+RSV vs. pcDNA+RSV: $p=0.0050$; SUR1+RSV vs. SUR2B+RSV: $p=0.0013$; SUR2B+RSV vs. pcDNA+RSV: $p=0.0018$). Cells expressing the mutant SUR1(M1289T), at which a single amino acid in transmembrane helix 17 was exchanged by the corresponding amino acid of SUR2B, gave similar data as pcDNA-controls (SUR1(M1289T)+RSV vs. pcDNA+RSV: $p=0.39$). Staining with Hoechst 33258 and determination of DEVD-caspase substrate cleavage revealed that resveratrol-induced cell detachment was associated with nuclear condensation and fragmentation and with increased caspase 3-like activity. Again, these effects were much more intensive with cells expressing the SUR1 isoform. Treatment with etoposide (ETO) however, showed no difference between SUR1-cells or pcDNA controls concerning all parameters tested in this study. For both cell lines, the extent of the etoposide-induced cell detachment was nearly the same as for control cells after resveratrol-treatment, but significantly lower than SUR1-cells after incubation with resveratrol (SUR1+ETO vs. pcDNA+ETO: $p=0.87$; SUR1+ETO vs. pcDNA+RSV: $p=0.37$; SUR1+ETO vs. SUR1+RSV: $p=0.045$).
Conclusions: Expression of SUR1 in HEK293 cells markedly enhances the induction of apoptotic processes by resveratrol but not by etoposide. Therefore, expression of SUR1, but not of SUR2B or the mutant SUR1(M1289T), is able to influence induction of apoptosis and could possibly mediate regulation of β-cell mass by specific agents.

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Distinct effects of glucose and other modulators of K^+_{ATP} channels on $[Ca^{2+}]_c$ in single mouse α-cells
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Background and Aims: Stimulus-secretion coupling in pancreatic α-cells is poorly understood. Changes in the free cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) are implicated in the control of glucagon release, but the mechanisms by which secretagogues modify α-cell $[Ca^{2+}]_c$ remain highly controversial. These were studied here in single α-cells isolated from a mouse model expressing a fluorescent protein (FP) under the control of the glucagon promoter.
Materials and Methods: Single cells, obtained by dispersion of isolated islets, were cultured for 1-3 days on glass coverslips in RPMI medium containing 7 mM glucose (G). For $[Ca^{2+}]_c$ measurements, the cells were loaded with 1 μM fura-PE3 in the culture medium. The coverslips were then trans-

ferred on the stage of an inverted microscope. Only cells expressing the FP (easily recognized upon excitation at 490 nm) were studied. $[Ca^{2+}]_c$ was measured with an imaging system by exciting the cells alternatively at 340 and 380 nm and collecting the emitted fluorescence at 510 nm. The fluorescence of the FP did not interfere with that of fura-PE3. Patch-clamp (perforated mode) was used to measure the K^+_{ATP} current.
Results: All islet cells expressing the FP were α-cells, as shown by immunodetection of glucagon. As expected, α-cells responded by a large $[Ca^{2+}]_c$ rise upon stimulation by 10 μM adrenaline or 10 mM arginine. During perifusion with 0.5 mM G, $[Ca^{2+}]_c$ oscillated in 55% (47/86) of α-cells, but was low in the others. Application of 15 mM G to oscillating cells did not significantly change $[Ca^{2+}]_c$, whereas inhibition of ATP synthesis by 2 mM azide abolished $[Ca^{2+}]_c$ oscillations and decreased $[Ca^{2+}]_c$ to nearly basal levels. Patch-clamp recordings showed that α-cells possess a K^+_{ATP} current with similar characteristics to that of β-cells: a large activation by 250 μM diazoxide (Dz) + azide, and a robust inhibition by 250 μM tolbutamide (Tb). Closing K^+_{ATP} channels by addition of 10–500 μM Tb to a medium containing 0.5 mM G increased $[Ca^{2+}]_c$ in all α-cells. By contrast, increasing [G] from 0.5 to 15 mM in the continuous presence of 10 μM Tb decreased $[Ca^{2+}]_c$ by 40%. Opening K^+_{ATP} channels with 100 μM Dz abolished $[Ca^{2+}]_c$ oscillations occurring in 0.5 mM G, and lowered $[Ca^{2+}]_c$ to basal levels. Dz also fully reversed the effects of 10 μM Tb, and completely prevented 10 mM arginine from increasing $[Ca^{2+}]_c$. Inhibition of L-type Ca^{2+} channels with 1 μM nimodipine virtually abolished $[Ca^{2+}]_c$ oscillations in 0.5 mM G, and largely reversed the effects of 10 μM Tb (77%) or 10 mM arginine (89%).
Conclusion: In contrast with previous suggestions, our results show that opening and closure of K^+_{ATP} channels, or inhibiting mitochondrial metabolism with azide in α-cells change $[Ca^{2+}]_c$ in a similar way as in β-cells. However, contrary to β-cells, isolated α-cells are poorly responsive to glucose alone, which only slightly decreases $[Ca^{2+}]_c$. This suggests that glucose does not induce major changes in the ATP/ADP ratio in single α-cells and supports the hypothesis that inhibition of glucagon secretion by α-cells in situ mainly results from an indirect effect of glucose involving non α-cells. The rise in $[Ca^{2+}]_c$ elicited by agents such as Tb or arginine mainly depends on Ca^{2+} influx through L-type Ca^{2+} channels.

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Glucose inhibits glucagon release independently of K_{ATP} channels
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Background and Aims: The signal transduction underlying glucose inhibition of glucagon secretion from the pancreatic α-cells is not well understood. It has been argued that glucose paradoxically inhibits glucagon secretion by depolarizing the α-cell via inhibition of K_{ATP} channels. We instead proposed that glucose acts via a K_{ATP} channel independent mechanism by inhibiting a store-operated depolarizing current. By parallel recordings of the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) and membrane potential (MP) in α-cells and separate measurements of glucagon secretion from pancreatic islets we have now tested whether closure of K_{ATP} channels are required in the signal transduction leading to glucose inhibition of glucagon secretion.
Materials and Methods: Single cells were prepared from C57BL/6 mouse pancreatic islets. $[Ca^{2+}]_i$ and membrane potential were measured in parallel with the fluorescent indicators fura-2 and bis-oxonol, respectively, using a digital imaging technique. After each experiment the α-cells were identified by immunostaining. Glucagon secretion from mouse islets was measured by radioimmunoassay.
Results: Parallel recordings of MP and $[Ca^{2+}]_i$ revealed that only 21% of the α-cells reacted to 0.5 mM of the depolarizing K_{ATP} channel inhibitor tolbutamide with elevation of $[Ca^{2+}]_i$ in presence of 1 mM glucose. Also a slight depolarization by raising the extracellular K^+ concentration from 4.8 to 8 mM caused a $[Ca^{2+}]_i$ response in 18% of the α-cells. However, combining 8 mM K^+ with 0.5 mM tolbutamide resulted in sustained elevation or oscillations $[Ca^{2+}]_i$ in all of 57 α-cells. Neither the $[Ca^{2+}]_i$ oscillations nor MP were affected by the N-type Ca^{2+} channel antagonist ω-conotoxin whereas the L-type Ca^{2+} channel blocker nifedipine inhibited the $[Ca^{2+}]_i$ response and hyperpolarized all of 12 α-cells. The depolarization and elevation of $[Ca^{2+}]_i$ in response to 8 mM K^+ and tolbutamide were inhibited by glucose with maximal effect already at 5 mM of the sugar. Glucagon secretion in 1 mM glucose was inhibited by either tolbutamide or 8 mM K^+ but not when combining tolbutamide with 8 mM K^+. Rise of glucose from 1 to 20 mM had a similar inhibitory effect on glucagon secretion both under control conditions and in the presence of tolbutamide + 8 mM K^+.
Conclusion: K_{ATP} channels in mouse α-cells are functionally active but their closure does not depolarize sufficiently to activate voltage-dependent Ca^{2+} influx. The rise of $[Ca^{2+}]_i$ in depolarized α-cells is due to activation of L-type Ca^{2+} channels. Glucose hyperpolarizes the α-cells, inhibits $[Ca^{2+}]_i$ signaling and glucagon secretion independently of the K_{ATP} channels. The inhibition of glucagon secretion by small depolarization by tolbutamide alone or 8 mM K^+ could not be explained by corresponding effects on α-cell Ca^{2+} signaling and may involve paracrine actions.
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Gluco- and lipotoxicity to beta cells

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Exendin-4 administration in mice stimulates expression of protein kinase A and reduces apoptosis in islets

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Background and Aims: Glucagon-like 1 peptide (GLP-1) potently augments glucose stimulated insulin secretion (GSIS) and has also been suggested to stimulate growth and differentiation of islets, as well as exerting anti-apoptotic effects. These effects are seen in association with elevation of cAMP and activation of protein kinase A (PKA). We recently demonstrated that GLP-1 receptor activation by exendin-4 administration in mice improves GSIS and increases the islet sensitivity to cAMP elevation. This could be explained by an additional effect of exendin-4 also to increase the expression of PKA. In this study we have therefore explored whether long-term exendin-4 treatment of normal and insulin resistant mice affects islet expression of the catalytic and regulatory unit of PKA (PKAcat and PKAreg) and whether this is associated with perturbation of islet markers for proliferation and apoptosis.
Materials and Methods: Female C57BL/6J mice were fed a normal diet (11% fat by energy; LF) or a high-fat diet (58% fat; HF) for 8 weeks. The high-fat feeding results in insulin resistance, defective islet compensation and impaired glucose tolerance. For exendin-4 treatment, mice were injected intraperitoneally once daily with 2 nmol/kg for 14 days; thereafter islets were isolated and analyzed for insulin secretory response to glucose and cAMP elevation by forskolin, expression of PKAcat and PKAreg, protein kinase B (Akt1/PKBa) and PDX-1 (by Western blot), and Caspase 3/7 activity.
Results: Islets from exendin-4-treated normal and high-fat fed mice displayed elevated insulin response to cAMP stimulation (LF: 2.5 ± 0.3 vs. 1.4 ± 0.1 ng/h/islet, P=0.001 and HF: 2.9 ± 0.3 vs. 1.9 ± 0.2 ng/h/islet, P=0.011). Interestingly, islets from high-fat fed mice displayed elevated sensitivity to cAMP elevation with augmented insulin secretion compared to normal islets (P=0.017). This was associated with increased islet expression of PKAcat 1.8 fold and PKAreg 1.6 fold compared to normal islets. In normal mice, exendin-4 treatment increased islet expression of PKAcat 2-fold and PKAreg 1.6-fold compared to control islets. In high-fat fed mice, PKAcat was increased 1.2 fold while PKAreg was similar to the high-fat fed control group. In normal mice, exendin-4 treatment decreased caspase 3/7 activity (371 ± 9 vs. 308 ± 15 AU/mg protein, P=0.01), whereas in insulin resistant mice, no significant effect in caspase 3/7 activity was observed after 14 days of exendin-4 treatment (323 ± 15 vs. 316 ± 7 AU/mg protein). The islet protein expression of the proliferative markers PDX-1 and Akt1/PKBa were unaltered by exendin-4 treatment in both normal and in insulin resistant mice.
Conclusion: Two weeks of GLP-1 receptor activation by exendin-4 upregulates the islet expression of PKA in both normal and insulin resistant mice. This was associated with reduced islet apoptosis in normal mice, but not in high-fat fed insulin resistant mice. Exendin-4 administration did not affect expression of the proliferative markers PDX-1 and Akt1/PKBa. This study demonstrates that exendin-4 administration to mice results in augmented insulin secretion through upregulation of PKA expression and that exendin-4 reduces islet apoptosis by an effect, which is prevented by high-fat feeding.

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The DPP-4 inhibitor vildagliptin increases pancreatic beta cell mass in rodents

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Background and Aims: The incretin hormone glucagon-like peptide-1 (GLP-1) has been shown to enhance pancreatic beta cell mass via either by increasing islet cell replication and neogenesis or by decreasing apoptosis. The current study addressed whether daily administration of the incretin enhancer vildagliptin could also increase beta cell mass, measured by pancreatic immunohistochemistry and morphometric analysis, in neonatal rats and streptozotocin (STZ)-induced beta cell injured mice.
Materials and Methods: Neonatal rats were orally dosed once daily with vildagliptin (60 mg/kg/day) or vehicle (control) from days 1 to 21 and beta cell turnover was measured on days 7, 21 and 28. In the other study, mice were injected intraperitoneally (IP) daily on days 1-5 with 60 mg/kg STZ and treated with vildagliptin (30 mg/kg/day, oral) or the GLP-1 agonist exendin-4 (0.42 mg/kg, IP) or vehicle starting 5 days prior to STZ treatment through day 15, and were analyzed on days 6, 16 and 25 for oral glucose tolerance, islet morphometrics, and pancreatic gene expression.
Results: In neonatal rats on day 7, vildagliptin increased (8-fold) beta cell replication (BrdU-positive islets) and decreased (64%) apoptosis (Apoptag-positive cells) compared to controls. On day 21 vildagliptin increased beta cell mass by 50% with a 23% increase of pancreatic insulin content. Beta cell mass remained 43% elevated on day 28 (1 week following the end of treatment). In the next study, STZ treatment in mice produced progressive loss of beta cell mass (38% to 66% from days 6 to 25) and simultaneously increased fasting blood glucose (FBG) producing glucose intolerance. Both vildagliptin and exendin-4 decreased FBG and improved glucose tolerance equally at day 16. This improvement in glucose tolerance was sustained 10 days after discontinuation of the treatment. While neither drug had an effect on beta cell mass, both vildagliptin and exendin-4 increased pancreatic PDX-1 mRNA levels on day 6 by 63% and 76%, respectively and elevated the number of ductal islets on day 16 by 60% and 45%, respectively.
Conclusions: These data from rats and mice demonstrate that the incretin enhancer vildagliptin can increase beta cell mass via enhanced cell replication or neogenesis and by decreased islet cell apoptosis in rodents. Furthermore, oral inhibition of DPP-4 is equally effective as an injected GLP-1 analog exendin-4 on glycemic control in a mouse model of beta cell injury.

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Evidence for a role of oxidative stress in both β cell lipotoxicity and glucotoxicity

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Background and Aims: An important mechanism which may be involved in the pathogenesis of type 2 diabetes is prolonged elevation of plasma free fatty acids (FFA) and glucose, which induce insulin resistance and can impair both β cell function and mass (β cell lipotoxicity and glucotoxicity). Whether the mechanisms responsible for lipo and glucotoxicity are identical, overlapping or distinct is still unclear. Oxidative stress has been implicated in β cell glucotoxicity, which is in many aspects similar to lipotoxicity. The objective of our study was to investigate the role of oxidative stress in β cell lipotoxicity and glucotoxicity.
Materials and Methods: To determine whether antioxidants prevent β cell lipotoxicity we infused iv oleate (OLE 1.3 μM/min) for 48 h with or without the antioxidant taurine (TAU 2.76 μmol/kgmin) in Wistar rats. Saline-treated rats (SAL) were used as control. After the 48 h infusion we performed hyperglycemic clamps to evaluate glucose stimulated insulin secretion (GSIS) *in vivo* or we isolated islets to evaluate insulin secretion *ex vivo*. To further evaluate the role of oxidative stress in β cell lipotoxicity we examined the response to *in vitro* exposure to OLE (0.4 mM in BSA) and TAU (1 mM) in the MIN6 β cell line. In both islets and β cell line we measured reactive oxygen species (ROS) by the dihydrofluorescein method.
Results: The insulin and C-peptide responses to the hyperglycemic clamp which were reduced by OLE ($p<0.01$ OLE vs SAL) were completely restored by coinfusion of TAU (p=NS SAL vs OLE+TAU or TAU). Similar to the findings *in vivo*, GSIS in isolated islets was impaired by OLE ($p<0.01$) but was restored by TAU (SAL=1.6 ± 0.2 pmol/islet/h OLE=1.1 ± 0.1 $p<0.01$ vs SAL; OLE+TAU=1.6 ± 0.2 TAU=1.7 ± 0.2 p=NS vs SAL). *In vivo* treatment with an equimolar dose of another antioxidant, NAcetylcysteine (NAC), was equally effective to TAU in abolishing the FFA induced decrease in GSIS of isolated islets. In the MIN6 cells, exposure to 48 h OLE impaired the insulin response to high glucose and addition of TAU restored secretion. RT-PCR of RNA from MIN6 cells treated with OLE showed induction of antioxidant (Cu/Zn-SOD) and inflammatory (COX-2) genes, an effect prevented by TAU. Furthermore, TAU abolished the increase in ROS induced by OLE in MIN6 cells, and both TAU and NAC had the same effect in isolated islets. To determine the role of antioxidants in glucotoxicity we infused iv glucose (20 mM) to obtain hyperglycemia, with or without TAU and NAC for 48 h in the same animal model and measured GSIS of isolated islets. Similar to OLE hyperglycemia impaired GSIS of isolated islets, however, neither TAU nor NAC were able to prevent this effect. Doubling the amount of TAU was also ineffective. To further investigate the effect of antioxidants in our model of glucotoxicity we coinfused glucose with tempol (25 mg/kg/h), a superoxide dismutase mimetic which restored GSIS (p=NS vs SAL) and

ROS levels in isolated islets. None of the antioxidants used had any effect on GSIS when infused alone in any experimental condition.
Conclusion: Our data strongly suggest that oxidative stress plays a role in the impairment of β cell function induced by prolonged elevation of both FFA and glucose, however, different antioxidants are effective in β cell lipotoxicity and β cell glucotoxicity suggesting that the amount and/or type of oxidative stress is different.
Support: CIHR

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Elevated circulating FFAs levels causing the pancreatic islet cell dysfunction through oxidative stress
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Background and Aims: Circulating free fatty acids (FFAs) are elevated in subjects with metabolic syndrome and type 2 diabetes. Studies in vitro showed that prolonged exposure to FFAs had caused reduced glucose stimulated insulin secretion (GSIS) and apoptosis of human pancreatic beta-cells, but the results conducted in vivo were rare and not consistent. In an effort to better understand the phenomenon of lipotoxicity in beta-cells, we evaluated the effects of a 4-days infusion of a triglyceride emulsion in Sprague-Dawley (S-D) rats on basal and GSIS in pancreatic beta-cell and investigated some of the possible mechanisms.
Materials and Methods: Three groups S-D rats underwent 2~4 days infusions: Controls (C, n=10): saline alone; FFA (n=15): 20% intralipid + heparin (intralipid : heparin = 1 ml : 40IU), infusion rates varied from 0.6–1.2 ml/h to maintain circulating FFA concentration at 2~3 times above the baseline; N-acetylcysteine +FFA (NAC+FFA, n=5):N-acetylcysteine (300 mg/kg.d) and intralipid at the same infusion rates (0.6–1.2 ml/h).The intravenous glucose tolerant test(IVGTT) was performed at the end of 4-days infusion. The pancreatic tissues were harvested at the end of 2-days and 4 days infusion and the isolated pancreas were perfused with Kerbs-Ringer bicarbonated (KRB) buffer contained with 3 mM and 16.7 mM glucose respectively.
Results: Basal plasma FFAs levels were similar in the various groups and they elevated 2.3 ~2.8 times in FFA and NAC+FFA groups after infusion. In FFA group the insulin secretion was inhibited and the plasma glucose was higher during IVGTT (Table 1). The basal insulin secretion by isolated pancreas was higher in FFA group after 2 days intralipid infusion (FFA 2-days:1154 ± 376uIU, C:544 ± 125uIU, P<0.05), but the phenomenon disappeared after 4 days infusion (FFA 4-days: 479 ± 100uIU, C:544 ± 125uIU, P>0.05). The GSIS (perfused with 16.7 mM glucose) after 2-days infusion were similar in FFA and C group (FFA: 4-days:896 ± 125 uIU vs.C:735 ± 204uIU, P>0.05), but GSIS were significantly lower after 4-days intralipid infusion (FFA: 4-days: 447 ± 50uIU, C: 4-days: 735 ± 204uIU, P<0.05). In NAC+FFA group, the GSIS was partly recovered (FFA+NAC: 714 ± 75uIU, P<0.05).

Table 1. serum glucose and insulin concentration during IVGTT after 4 days infusion.

Time (min)		0	1	5	10	30	60
Glucose (mmol/L)	C	5.1 ± 1.1	16.9 ± 1.8	12.9 ± 0.5	10.3 ± 1.6	9.1 ± 2.9	8.4 ± 2.1
	FFA	5.0 ± 0.4	16.9 ± 2.3	15.2*± 2.0	12.9 ± 2.6	11.7 ± 1.7	8. 8 ± 1.1
Insulin (mIU/L)	C	18.0 ± 5.1	84.3 ± 20.3	41.3 ± 7.4	32.7 ± 3.6	26.1 ± 3.0	23.6 ± 2.6
	FFA	14.1 ± 4.4	25.0*± 2.6	33.0 ± 8.1	25.0 ± 7.4	15.9*± 2.9	20.3 ± 4.5

* P<0.05 or less for difference vs. value at control group.

Conclusion: Short-time elevation of FFAs (2 days) in rats produce the beta cell inappropriate hypersecretion at low glucose concentrations, and an insufficient response to increased glucose, while a prolonged exposure to FFAs (4 days) causes reduced both basal and glucose stimulated insulin secretion in vitro and in vivo. Antioxidant acetylcysteine may partly mitigate impaired beta-cell function produced by elevated FFA concentration. Elevated circulating FFAs levels may contribute to causing the abnormalities of pancreatic islet cell function through active oxidative stress or oxidative stress-sensitive signaling pathway.
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Regulation of beta-cell uncoupling protein-2 expression and cell viability by free fatty acids and leptin
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Background and Aims: Uncoupling protein-2 (UCP-2) has been shown to be involved in beta-cell dysfunction, possibly through its ability to regulate insulin secretion, reactive oxygen species levels and apoptosis. We have previously reported that glucose increases and leptin decreases the levels of UCP-2 expressed in human islets. Free fatty acid (FFA) induced beta-cell dysfunction (lipotoxicity) is known to act synergistically with glucose toxicity to destabilise the beta-cell.
Materials and Methods: The effects of FFA's (oleate and palmitate), cytokines (TNF-alpha and IL-1beta) and leptin on UCP-2 mRNA and protein expression (measured by RT-PCR and western blotting respectively) and cell viability (measured by a modified MTS assay) were investigated in the clonal rodent beta-cell line BRIN-BD11.
Results: Oleate caused a marked increase in UCP-2 mRNA expression at 5.5 mM glucose (p<0.001), but this was reversed when cells were incubated with oleate:palmitate. At 22 mM glucose, TNF-alpha caused a decrease in expression (p=0.02). We confirmed the inhibitory effect of leptin on UCP-2 mRNA and protein expression in BRIN-BD11 cells and demonstrate that leptin reduced the toxic effects of exposure to long term glucose.
Conclusion: FFA and cytokines appear to differentially regulate UCP-2 expression in the BRIN-BD11 cells with oleate being a strong inducer of UCP-2 expression at low glucose. Leptin inhibits UCP-2 expression at high glucose but protects from glucose toxicity *in vitro* in the beta-cell.

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Effect of high fat diet on beta cell mass, proliferation, neogenesis and transmissibility to the progeny
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Background and Aims: Adequate nutrition during fetal development is a critical factor contributing to the etiology of obesity and diabetes in adult life both in humans and animals. Aim of our study was
1) to investigate effects of high fat diet in adult mice (β-cell mass, proliferation, neogenesis and apoptosis; insulin secretion and sensitivity) and reversibility after suspension of the HF diet;
2) to verify effects of maternal metabolism induced by HF diet before and during pregnancy on 1st (F1) and 2nd (F2) generation of mice;
3) to evaluate effects of HF diet on the metabolic imprinting (MI) in F1 and possible transmission to the progeny.
Materials and Methods: Aim 1: 50 female mice were randomized at ~2 months of age into 3 groups; one group received a standard chow diet (CD) for 2 months; another received a high-fat (HFD) diet for 2 months; the last one received a high-fat (HFD) diet for 2 months and then a CD for 2 months (HFDCD). Animals underwent an intraperitoneal glucose tolerance test (IPGTT) 2 months before and after the beginning of the high-fat diet and 2 months after suspension of the diet. Their pancreas was removed for immunohistochemical analysis.
Aim 2: 40 females mice were bred with males and divided into 2 groups, 1 fed by chow diet (CD) and 1 fed by High fat diet (HFD). On the 4th day after the birth, the obtained progeny (F1) was fed by an intragastic cannulas, and randomly received a HFD or CD. Since the postnatal day 24 all F1 mice were fed with normal adult CD. On day 60, F1 mice underwent an IPGTT. Pancreas was removed for immunohistochemistry. The remaining F1 females were bred with normal male mice, and F2 were obtained. F1 foster mothers nursed F2 until day 24, when they were weaned on laboratory CD. On day 60 F2 mice underwent an IPGTT. Pancreas was removed for immunohistochemistry.
Results: Aim 1: HF diet induced increased glucose levels, β cell mass and replication, endocrine cell neogenesis; no effects were observed in the apoptotic rate. These effects were partially reversible after suspension of HF diet.
Aim 2: The maternal alterations due to HF diet induced hyperglycemia after the glucose load and increased endocrine neogenesis in F1. The following progeny (F2) showed increased basal and post load glucose levels due to a reduced β cell replication.
Aim 3: The effect of HF diet in the suckling period determined increased β cell replication, increased glucose levels after the glucose load and

endocrine neogenesis. F1 showed increased glucose levels only after the glucose load, and a reduced β cell replication. The combined effect of maternal metabolism and MI determined increased glucose levels after the load, increased β cell mass and replication, and increased endocrine neogenesis. F2 showed increased basal and after the load glycemic levels and increased β cell mass.
Conclusions: HF diet determines significant, reversible alterations in adult mice. In the F1, the MI can determine hyperglicemia, increased replication, endocrine neogenesis, while β cell mass seems to be influenced more by maternal factors during fetal life combined with MI. Only the direct effect of HF diet affects β cell replication. The transmission to the progeny seems to depend on the concomitant effect of maternal influence and MI.

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Reduced pancreatic insulin content in offspring of rats fed high saturated fat during pregnancy
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Background and Aims: Nutritional imbalance during pregnancy can lead to developmental abnormalities in the fetus, increasing risk of cardiovascular dysfunction and diabetes later in life. High fatty acid concentrations can induce beta cell apoptosis and alter secretory function in islets and, if experienced in fetal life, could impair beta cell development with long-term consequences for glucose regulation. The aim of this study was to examine whether high fat feeding during pregnancy influences beta cell development in the offspring at birth and at weaning.
Materials and Methods: Female Sprague Dawley rats were fed for 10 days before mating, throughout pregnancy and during lactation on control laboratory chow (5.3% fat), or chow supplemented with 24% fat as lard, saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), or polyunsaturated fatty acid (PUFA). Pancreases were obtained from offspring at 48 hours and 21 days after birth and either snap-frozen or formalin-fixed and embedded in paraffin. Frozen pancreas specimens (8–17 samples per group) were dissected free of contaminating fat and other tissue, weighed and extracted in acid ethanol for analysis of pancreatic insulin content by ELISA. Fixed pancreas samples at 48 h were sectioned and labelled for insulin by immunofluorescence and double-stained for apoptotic nuclei by TUNEL as a measure of beta cell apoptosis.
Results: At 48 hours post partum, pancreatic insulin contents of offspring of rats fed PUFA during pregnancy were significantly elevated above those fed control diets (235.2 ± 16.5 vs 176.4 ± 17.6 ng/mg pancreas weight; $p<0.025$), whereas pancreases in other groups were not significantly different from control samples. By 21 days of life, a significant reduction in pancreatic insulin content was observed in offspring of rats fed lard (45.8 ± 17.6 ng/mg; $p<0.0001$) or SFA (81.2 ± 10.2 ng/mg; $p<0.0002$) compared to control (162.9 ± 16.2 ng/mg). TUNEL-positive nuclei were detected in insulin-positive cells in offspring of lard and SFA fed dams.
Conclusion: The results demonstrate that maternal diets rich in saturated fats during pregnancy and suckling cause significant decreases in pancreatic insulin levels in their offspring by the time of weaning. Loss of insulin occurs during the period of pancreas remodelling in the first three weeks of life and is associated with beta cell apoptosis. A maternal diet rich in saturated fat may therefore have deleterious effects on pancreas development in the offspring and may increase risk of diabetes development later in life.
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Glucotoxicity explored by proteomic analysis of INS-1E cell mitochondria
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Background and Aims: Hyperglycemia has been connected with development of impaired glucose-stimulated insulin secretion (GSIS) and type 2 diabetes mellitus. Although specific β-cell proteins have been coupled to deterioration of GSIS, the mechanisms by which elevated glucose concentrations contribute to secretory disturbance (glucotoxicity) are still unclear. One explanation for the difficulty in dissecting causes of glucotoxicity is its polygenic etiology. This prompts the use of approaches capable of determining expression profiles rather than single gene products. The aim of the present study was to investigate mechanisms of β-cell glucotoxicity by analyzing changes in complex protein patterns with surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI TOF-MS). Such protein patterns were obtained from mitochondria isolated from INS-1E cells cultured at normal and elevated glucose concentrations.
Material and Methods: INS-1E cells were cultured for 5 days in 24-well plates and flasks at 5.5, 11, 20 or 27 mM glucose. After culture, plated cells were exposed to 3 or 15 mM glucose for 30 min and insulin released measured by ELISA. Cells in flasks were harvested and mitochondria isolated by subcellular fractionation. Mitochondrial proteins were extracted, applied on anionic protein arrays (SAX2) and protein profiled by SELDI TOF-MS. Analysis was performed and 3 differentially displayed peaks, corresponding to proteins with masses 4.1 kD, 7.0 kD, 12.6 kD, were chosen for further investigation. An anionic exchange column was used for fractionation of the crude mitochondrial sample and the biomarker containing fractions were desalted by a 3 kD spin column. Fractionated samples were subjected to one-dimensional SDS-PAGE (12%). Gel pieces were excised and proteins passively eluted. Eluates were re-applied on SAX2 arrays and analyzed by SELDI TOF-MS.
Results: Insulin release was measured in the presence of 3 or 15 mM glucose. Whereas insulin release increased from 2 to 15 and 2 to 25 pmol/well*30 min ($p<0.05$) from INS-1E cells cultured in the presence of 5.5 or 11 mM glucose respectively, no change in insulin release was observed from cells cultured at 20 or 27 mM glucose. Protein profiles of mitochondria, isolated from INS-1E cells cultured at the four different glucose concentrations, showed about 80 peaks (s/n=4, peak percentage=20%). Three differentially expressed proteins were chosen for further analyses: 4.1 kD with 8-fold increase ($p<0.05$), 7.0 kD with 4-fold increase ($p<0.05$) and 12.6 kD with 4-fold increase ($p<0.05$) in intensity for cells cultured at 27 mM glucose compared to 5.5 mM glucose. After fractionation of the crude mitochondrial sample, the 12.6 kD protein was obtained in the pH 3 fraction and the other two proteins in the organic fraction. The proteins of the fractions are to be further separated by one-dimensional gel electrophoresis after which relevant gel pieces will be excised blindly and proteins passively eluted. Successful elution will be followed by trypsin digestion and peptide mass fingerprinting.
Conclusion: The proteomic approach used to address causes of glucotoxicity revealed multiple differentially displayed mitochondrial proteins, when comparing mitochondrial profiles obtained from INS-1E with normal GSIS to profiles obtained from INS-1E showing signs of glucotoxicity. Completion of the identification work is expected to give new insights into the molecular mechanisms of glucotoxicity.

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Chronic mild hyperglycemia is associated with an inflammatory islet reaction in a spontaneous model of type 2 diabetes (T2D), the Goto-Kakizaki (GK) rat
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Background and Aims: The GK rat spontaneously develop mild hyperglycemia from 3 to 4 weeks onwards without obesity. In 4-month-old diabetic GK rats, total β-cell mass is decreased by 60% in parallel to pancreatic insulin stores, while 2 populations of islets are observed: 1) large islets with spots of heterogenously insulin-stained cells intermingled with fibrosis and 2) small (normal) islets with heavily stained β cells and normal architecture. Such alterations also exist in other spontaneous rat T2D models (Zucker, OLEFT and Torii). Here, we present a complementary approach using gene expression analysis and immunohistochemistry to identify potential inflammatory reaction that might lead to islet fibrosis and beta-cell impairment in the GK model of T2D.
Materials and Methods: Affymetrix micrroarrays were used to compare gene expression in islets isolated from 4-month-old GK rats and control age-matched Wistar rats, from which the GK strain was originally selected. Immunohistochemistry was performed on 1- and 4-month-old Wistar and GK cryostat pancreas sections for proteins related to extracellular matrix (ECM), different types of macrophages, cytokines, vascularization and a Schwann cell marker (glial fibrillary acidic protein or GFAP).
Results: Affymetrix micrroarrays showed overexpression of genes for molecules that belong to: 1) the extracellular matrix (ECM)-related proteins and cell adhesion molecules, particularly, collagen I and III, fibronectin, osteopontin and TIMP-1 (tissue inhibitor of metalloprotease-1); 2) the inflammatory/immune response, particularly MHC class II and immunoglobulins; 4) the oxidative stress. The overexpression of some of these genes has been confirmed by quantitative RT-PCR. Immunohistochemically, compared to 4-month-old control Wistar islets, GK islets showed: 1) diffuse intra-islet labeling for collagen I and III, fibronectin, with an almost complete disappearance of intra-islet vascularization, as confirmed by von

Willebrand factor (vWF) and laminin labeling; 2) a more marked intra-islet labeling of osteopontin, an adhesion and chemotactic protein linked to inflammation; 3) signs of intra-islet gliosis as demonstrated by the presence of GFAP$^+$ cells that normally surround the islets; 4) more vWF$^+$ and TIMP-1$^+$ peri-islet vessels; 5) accumulation of various populations of macrophages (MHC class II$^+$, ED1$^+$ and ED2$^+$) around some islets and/or at their vascular-ductal pole; 6) more intense TNFα (tumor necrosis factor-α) labeling of endocrine cells. No fibrosis nor vessels anomalies were observed, at diabetes onset, in 1-month-old GK islets.
Conclusion: These data shed light on the islet inflammatory process that is associated with the chronic, even mild, hyperglycemia of T2D GK rats. They also suggest that islet inflammation might be a common pathway leading to diabetes (whether of type 2 or 1)-induced beta-cell impairment, probably via cytokine-related events.

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Inhibitory effects of suppressor of cytokine signalling-3 on tumor necrosis factor-alpha induced signalling in pancreatic beta cells
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Background and Aims: Tumor necrosis factor-alfa (TNFa) is a proinflammatory cytokine which in synergy with interleukin-1-beta (IL-1b) and/or interferon gamma (IFNg) is cytotoxic to insulin producing pancreatic rat, mouse and human beta cells *in vitro* and is suggested to contribute to the specific beta cell destruction in type-1 diabetes mellitus (T1DM). During the pathogenic process leading to T1DM TNFa, IL-1b and IFNg are secreted by islet infiltrating macrophages and activated T-cells. Suppressor of cytokine signalling-3 (SOCS-3) is an intracellular negative regulator of cytokine signalling known to inhibit JAK-STAT signalling and to be up-regulated by the pro-inflammatory cytokines IL-1b, IFNg and TNFa in a cell-dependent manner. Recently, SOCS-3 has been demonstrated to inhibit IL-1b signalling by inhibition of mitogen-activated protein kinases (MAPKs) and nuclear factor kappa-B (NFkB). Like IL-1b, TNFa signals via MAPK and NFkB pathways and therefore the aim of this study was to investigate whether SOCS-3 also inhibits TNFa-induced signalling in beta cells and if SOCS-3 expression is induced by TNFa in beta cells.
Materials and Methods: In this study we used primary rat beta cells transduced with adenovirus encoding SOCS-3 and a beta cell line with doxycycline-inducible SOCS-3 expression. The effect of SOCS-3 on TNFa-induced signalling was investigated at the level of 1) MAPK phosphorylation and inhibitory kappa-B (IkB) degradation by use of Western Blotting, 2) NFkB DNA binding by use of Electrophoretic mobility shift assay and 3) NFkB-dependent gene transcription by use of Gene reporter assay. Furthermore, TNFa-induced SOCS-3 expression was investigated in primary beta cells by Real-Time RT-PCR.
Results: In the beta cell line and the primary rat beta cells SOCS-3 inhibited phosphorylation of the MAPKs JNK, p38 and ERK as well as IkB degradation. Furthermore, SOCS-3 inhibited TNFa-induced NFkB DNA-binding and NFkB-dependent gene transcription in the beta cell line. Finally, TNFa induced a clear transient expression of SOCS-3 peaking after 1–2 hrs in primary rat beta cells.
Conclusion: These results indicate that SOCS-3 inhibits signalling pathways initiated by TNFa and that TNFa itself induces the expression of SOCS-3 in beta cells suggesting that SOCS-3 might be a candidate therapeutic target for protection of beta cells.

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Characterization of the pattern of NF-kB activation in insulin-producing INS-1E cells
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Background and Aims: The cytokine IL-1β activates the transcription factor NF-κB in beta-cells, and blocking NF-κB activation prevents cytokine-induced beta-cell apoptosis. Paradoxically, NF-κB has mostly anti-apoptotic effects in other cell types. The cellular effects of NF-κB depend on the duration, periodicity and degree of activity of the particular dimers involved. The aim of the present study was to compare the pattern of cytokine-induced NF-κB activation between insulin-producing cells (in which NF-κB is pro-apoptotic) and rat fibroblast (in which NF-κB is anti-apoptotic) in order to clarify the reasons behind the pro-apoptotic effects of NF-κB in pancreatic beta-cells.
Materials and Methods: Rat insulin-producing INS-1E cells were exposed to either IL-1β (100U/ml) or TNF-α (1000U/ml), while rat fibroblast 208F cells were exposed to IL-1β (100U/ml) in time course experiments. Cell viability was evaluated by HO 342 and propidium iodide. NF-κB activation was examined by EMSA. Temporal expression of IκB isoforms was examined by real time RT-PCR and Western blot analysis, while intensity of IL-1β-induced NF-κB activation was analyzed using a NF-κB-luciferase reporter construct.

Results: NF-κB activation was induced in INS-1E cells after exposure to IL-1β or TNF-α and in 208F cells after exposure to IL-1β. Exposure of INS-1E cells to IL-1β induced apoptosis (apoptotic index of 4.0; $p<0.01$ vs. control after 48 h) while TNF-α did not. IL-1β did not affect the viability of 208F cells. NF-κB activation showed an oscillatory pattern, with the peak of NF-κB activation observed after 10 min in IL-1β-treated INS-1E cells, while it occurred later (30 min) in TNF-α-treated INS-1E or in IL-1β-treated 208F cells. The p50 subunit was absent in the early NF-κB complexes formed in IL-1β-treated INS-1E cells, while p65/p50 was the predominant NF-κB dimer detected in IL-1β-treated 208F cells. IL-1β-treated INS-1E cells had higher IκBα mRNA expression than TNF-α-treated INS-1E cells (2-3 fold higher from 30 min until 1.5 h) or IL-1β treated 208F (6–30 fold higher from 30 min until 8 h). The IκBα protein was degraded after 10 min in IL-1β-treated INS-1E cells, while degradation was less intense and detectable only after 30 min in TNF-α-treated INS-1E and IL-1β-treated 208F cells. IL-1β induced a similar profile of IκBβ and IκBε mRNA expression and protein degradation in INS-1E and 208F cells but with higher intensity in IL-1β-treated INS-1E cells. Expression of the mRNAs for the NF-κB target genes MCP-1, Fas and iNOS was higher in INS-1E cells exposed to IL-1β than TNF-α (2-30 fold), while in 280F cells exposure to IL-1β induced only MCP-1 expression. The intensity of NF-κB activation, as judged by the NF-κB-luciferase reporter assay, was higher in IL-1β-treated INS-1E cells (9.8 fold) than in TNF-α-treated INS-1E cells (2.3 fold) or in IL-1β-treated 208F cells (1.2 fold).
Conclusion: The pattern of IL-1β-induced NF-κB activation in INS-1E cells is different from the pattern induced by TNF-α in these cells, or by IL-1β in 208F cells. These differences are characterized by: 1. An earlier and stronger NF-κB activation in IL-1β-treated INS-1E cells; 2. A differential composition of NF-κB dimers in the activated complex; 3. A differential (quantitative and qualitative) expression of NF-κB target genes.
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Gadd45beta inhibits proapoptotic JNK signaling in pancreatic beta-cells exposed to IL-1beta
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By the induction of apoptosis in pancreatic β-cells IL-1β is believed to be an important mediator of the β-cell destruction leading to type 1 diabetes. IFN-γ and TNF-α potentiate the harmful effect of IL-1β. The apoptotic effect of IL-1β is primarily mediated by JNK, as inhibition of JNK activity completely blocks IL-1β induced β-cell apoptosis. Growth Arrest and DNA Damage-Inducible gene (gadd45)β has been reported to inhibit JNK activation via inhibition of the up-stream kinase MKK7.
Aim: To investigate gadd45β regulation by IL-1β in β-cells and non-β-cells and to evaluate the involvement of gadd45β in β-cell JNK signaling.
Methods: Quantitative RT-PCR using SYBR Green on RNA isolated from cells and islets exposed to cytokines and Western blotting. For overexpressing experiments cells were transfected using Fugene 6 and selected with G418.
Results and conclusion: IL-1β, IFN-γ and TNF-α induced a 10.9, 4.6 and 1.7 fold induction of gadd45β mRNA, respectively, in INS-1E cells (n=2). Time course experiments showed significant gadd45β induction by IL-1β from 0.5 h until at least 4 h with a peak of 11.7 at 2 h (n=4). The β-cell time-dependent gadd45β induction by IL-1β was confirmed using isolated islets, where IL1-β caused gadd45β mRNA induction within 0.5 h until at least 24 h with a peak 30.1 fold induction at 2 h (n=2). Preincubation of INS-1E with IL-1β and cyclohexamide caused a 3.8 fold superinduction over IL-1β treated levels (n=4). IL-1β caused a minor induction of gadd45β mRNA in two β-cell lines (INS-1E and βTC3) compared to IL-1β exposed NIH-3T3 fibroblasts (11 and 4 fold vs 120 fold, respectively) (n=3–4), indicating insufficient gadd45β induction by IL-1β in β-cells. By phospho-specific antibodies MKK7 was shown to be activated by IL-1β in INS-1E cells within 15 minutes and sustained until at least 2 h (n=3). As a preliminary evaluation of gadd45β involvement in β-cell JNK signaling, pools of overexpressing INS-1E and βTC3 cells (tranfection efficiency around 50%) were exposed to IL-1β. gadd45β overexpression reduced IL-1β induced JNK activation by 42% and 63% in INS-1E and βTC3 cells, respectively (n=2).
In conclusion, gadd45β is a primary response gene in β-cell IL-1β signaling and the identification of gadd45β as a novel β-cell JNK inhibitor with insufficient induction may explain the β-cell proapototic response to IL-1β.
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The effect of palmitate or pro-inflammatory cytokines on the expression of the NADPH oxidase component p47-phox in the clonal pancreatic cell line BRIN BD11
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Aims and Background: Changes in circulating levels of glucose and/or fatty acids (FA's) may lead to a loss of β-cell function in the pathogenesis of type 2 diabetes. FA's acutely stimulate the secretion of insulin while chronic exposure may induce apoptosis. Saturated FA's such as Palmitic acid (PA) have a detrimental effect on β-cells increasing the rate of apoptosis. Increased circulating levels of PA are associated with type 2 diabetes. PA has been reported to stimulate apoptosis in beta cells via ceramide generation and interference in pro-survival signalling pathways. However, it is possible that chronic exposure of β-cells to FA's may cause apoptosis by upregulating components of the NADPH oxidase, leading to ROS production and cellular damage. One of the key regulatory components of this enzyme is P47-phox which is activated on phosphorylation, causing translocation to the plasma membrane and activation of the oxidase complex. We evaluated the effects of 24 hour exposure of BRIN BD11 cells to 100 µ_ Palmitic acid on p47Phox expression. In addition we treated BRIN BD11 cells with a pro inflammatory cytokine cocktail (containing TNF-α, INF-γ and IL-1β) to determine their effects on P47phox expression.
Materials and Methods: BRIN-BD11 cells were cultured in the presence or absence of 100 µM PA or pro inflammatory cytokines for 24 hours and then 3 µg RNA was isolated for analysis via RT-PCR. Semi-quantitative RT-PCR was performed to measure the levels of expression of P47-phox mRNA. Protein was also isolated from BRIN-BD11 cells treated as above for western blot analysis utilising a specific P47-phox antibody.
Results: The expression of P47-phox protein was significantly ($p<0.05$) upregulated in the presence of palmitic acid. P47-phox mRNA and protein were both significantly ($p<0.05$) upregulated in the presence of the pro inflammatory cytokine cocktail.
Conclusions: Both palmitic acid and the pro-inflammatory cytokine cocktail are known to induce apoptosis in primary islet cells and β-cell lines such as BRIN BD11. Our results are indicative of a potentially novel mechanism for induction of apoptosis involving increased expression of a key regulatory component of the NADPH oxidase.

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IL-1β + IFN-γ alter the mRNA but not protein expression of Bcl-2 family members in the rat clonal β-cell line INS-1E
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Background: Interleukin (IL)-1β and Interferon (IFN)-γ are pro-inflammatory cytokines that have been proposed to be important effectors in the pathogenesis of Type 1 Diabetes. The molecular mechanisms involved in cytokine-induced β-cell dysfunction and death include activation of NF-κB and MAPK signalling, ER stress and mitochondrial events. The family of Bcl-2 proteins are important regulators of mitochondrial events leading to apoptosis and consists of both anti-apoptotic (Bcl-2 and Bcl-X_L) and pro-apoptotic members (Bid, Bax, Bak, and Bad). Overexpression of Bcl-2 has been shown to inhibit cytokine-induced apoptosis (Saldeen 2000) and microarray analysis has revealed that IL-1β + IFN-γ increase the expression of the pro-apoptotic members Bid and Bak and modified the expression of other Bcl-2 members in INS-1E cells (Kutlu B et al 2003). It remains to be determined whether protein expression or the activity of the Bcl-2 protein family is regulated by cytokines in β-cells.
Aims: To investigate the effects of IL-1β + IFN-γ exposure on the expression of selected Bcl-2 members in the clonal β-cell line INS-1E by RT-PCR and Western blotting. The dependence on nitric oxide (NO) was further investigated by including the inducible NO synthase inhibitor N^G-monomethyl-L-arginine (NMA).
Material and Methods: INS-1E cells were exposed to IL-1β + IFN-γ in the absence or presence of NMA for 1, 2, 4, 8, 12, and 24 h or 4, 8, 12, and 24 hours, respectively, before Real-Time PCR or immunoblotting were performed.
Results: Real-Time PCR (n=7) demonstrated that 12 and 24 h exposure to IL-1β + IFN-γ increased expression of the pro-apoptotic genes Bid, Bax, and Bak. The up-regulation of Bid and Bax, but not Bak, was NO dependent as it was inhibited by NMA. Expression of the anti-apoptotic gene Bcl-X_L, but not Bcl-2, was also increased in a NO dependent way. Preliminary

results on protein expression showed no changes in expression of Bax (n=6), Bcl-X_L (n=4), or Bcl-2 (n=2).
Conclusion: Cytokines caused an increase in mRNA levels but not protein expression levels of both pro-apoptotic and anti-apoptotic members of the Bcl-2 family. Taken together, these data indicate that changes of Bcl-X_L, Bcl-2, and Bax protein levels are not essential in cytokine-induced pro-apoptotic signalling. However, other Bcl-2 members may be cytokine regulated as may the activity of these proteins by post-translational modifications.
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Inhibition of histone deacetylases (HDAC) prevents cytokine-induced β-cell toxicity
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Background and Aims: Type 1 diabetes mellitus is an autoimmune disease characterised by an immune mediated elimination of the pancreatic β-cells. Cytokines such as IL-1β and IFNγ, released in this process induce β-cell apoptosis *in vitro* by NO-dependent and -independent mechanisms. IL-1β activates the NFκB pathway, which is involved in iNOS expression, NO formation, and apoptosis. NFκB has recently been shown to associate with histone acetyltransferases (HAT) and histone deacetylases (HDAC) which affect the activity of NFκB in a cell type specific manner. HAT activity is generally associated with an increase in gene transcription leading to "unwinding" of DNA sequences by acetylating the histones thereby making promoter regions accessible to transcription factors. Positive effects of HDAC inhibition has been obtained in other inflammatory diseases but the effect on β-cell cytotoxicity is not known. The aim of this project was to investigate whether HDAC inhibition could protect β-cells against cytokine-induced toxicity.
Materials and Methods: Clonal insulin producing cells, INS-1E, or whole rat islets were precultured with the HDAC inhibitors, suberoylanilide hydroxamic acid (SAHA) and Trichostatin A (TSA) followed by exposure to IL-1β and IFNγ. Effects on insulin secretion and NO formation were measured by competitive ELISA and Griess reagent, respectively, whereas Cell death detection ELISA was used to measure apoptosis. NFκB activation was investigated by Western blotting using antibodies against IκBα and phospho-IκBα, in addition to an electrophoretic mobility shift assay with an NFκB binding probe.
Results: SAHA and TSA reduced cytokine-induced decrease in insulin secretion and increase in NO-production and apoptosis. IL-1β induced a bi-phasic phosphorylation of IκBα with the 2nd peak being sensitive to HDAC inhibition. However, no effect was seen on IκBα degradation and NFκB DNA binding.
Conclusion: Inhibiting histone deacetylases prevents cytokine-induced β-cell apoptosis and impaired β-cell function via effects on NFκB-induced gene transcription down-stream of DNA binding and may involve an increase in anti-apoptotic gene expression.
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Effects of antioxidant enzymes on UCP-2 expression in insulin-producing cells
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Background and Aims: Uncoupling protein-2 (UCP-2) has arisen recently as an important factor of insulin release disturbances in the diabetes development. But due to its uncoupling abilities, it has also been postulated that UCP-2 may play a role in the clearance of reactive oxygen species. Therefore it was the aim of this study to investigate the effect of increased or diminished antioxidant enzyme activities on the UCP-2 expression in insulin-producing cells.
Materials and Methods: The antioxidant enzymes Mn-superoxide dismutase (MnSOD*sense*), cytoplasmic catalase (Cyto-Cat), mitochondrial catalase (Mito-Cat), CuZn-superoxide dismutase (Cu/ZnSOD) and glutathione peroxidase (GPx) were stably overexpressed or stably suppressed (Mn-superoxide dismutase, MnSOD*antisense*) in insulin-producing RINm5F cells. The expression of the UCP-2 gene was analysed by quantitative real time PCR and by Western blot analyses.
Results: In all analysed cell clones UCP-2 could be detected, but no expression of the UCP-1 and UCP-3 isoforms. Cells overexpressing catalase with a cytoplasmic or a mitochondrial targeting signal showed with a 3.3-fold (Cyto-Cat) and 6.9-fold (Mito-Cat) a significant increase of UCP-2 gene expression in comparison to control cells. In glutathione peroxidase overexpressing cells, the increase of the UCP-2 expression level was 6.3-fold increased which is comparable with that observed in Mito-Cat cells. Manipulation of the different SOD enzymes resulted in an 2.7-fold increase in the case of MnSOD*antisense*, 1.5-fold increase in the case of MnSOD overexpression and in a 2-fold increase in Cu/ZnSOD overexpressing cells. These changes were confirmed on the protein level by Western blots.
Conclusion: The results indicate that UCP-2 expression in insulin-producing cells is under the control of reactive oxygen species, especially by hydrogen peroxide. Very high UCP-2 expression levels were found in cells overexpressing the hydrogen peroxide detoxifying enzymes catalase and GPx, especially when they were expressed in the mitochondrial compartment. Since these overexpressing cells are protected well against cytokine toxicity, increased UCP-2 expression is apparently not deleterious under these conditions.

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Pro-inflammatory cytokine or LPS induced changes in nutrient metabolism and insulin secretion in a clonal pancreatic β-cell line

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Background and Aims: LPS acts as a strong stimulator of the immune system and is a potential trigger for the autoimmune destruction of the β-cells in Type I diabetes. LPS can bind to and activate specific classes of Toll-like receptors. Pro-inflammatory cytokines, products of immune stimulation, cause substantial changes in β-cell gene expression in vitro and in vivo. Expression of TLR2 and TLR4 receptors (Toll-like receptors) has recently been reported in islet and β-cells. Previous work has reported that the expression of genes encoding enzymes related to nutrient metabolism is a major target for up- or down- regulation by pro inflammatory cytokines, e. g. Glut-1, Glut-2, iNOS & GAD. This study has examined the changes in β-cell nutrient metabolism and insulin secretion in response to addition of the pro-inflammatory cytokines (IL1β, TNFα and IFNγ) or LPS. Key changes in β-cell nutrient metabolism may contribute to the phenomenon of a pro-inflammatory cytokine induced depression in insulin secretion. In contrast, LPS induced changes in β-cell nutrient metabolism may be stimulated through alternative signalling pathways and may contribute to increased insulin secretion.
Materials and Methods: BRIN-BD11 cells were incubated in the presence of pro-inflammatory cytokines at sub-lethal concentrations. Changes in glucose, glutamine and alanine consumption and end-product formation were determined by spectrophotometric assay. Glucose, alanine and glutamine are consumed at substantial rates by islet cells and some β-cell lines including BRIN BD11. Mitochondrial dehydrogenase activity was determined by the MTS assay.
Results: Glucose consumption in the presence of the pro inflammatory cytokine cocktail was increased twofold (20.18 ± 1.31 to 40.26 ± 1.97 mmoles/mg protein, $p<0.001$). However, insulin secretion decreased (0.052 ± 0.004 to 0.017 ± 0.003 μg/10^6 cells/24 hours $p<0.0001$). Glutamine and Alanine consumption decreased (12.42 ± 2.31 to 7.82 ± 1.14 μmoles/mg protein $p<0.05$ and 9.83 ± 2.43 to 3.85 ± 4.25 μmoles/mg protein $p=0.061$ resp.). Glucose consumption and MTS reduction (mitochondrial activity) were increased in a dose-dependent manner by the pro-inflammatory cytokine cocktail. In contrast, nutrient induced insulin secretion was significantly increased after incubation with 100ng/ml LPS (14.18 ± 4.30 to 20.14 ± 4.60 ng/10^6 cells/20 mins, $p<0.05$), although glucose, glutamine and alanine consumption were unchanged. Glutamine consumption increased after incubation with the higher concentration of LPS 10,000ng/ml (0.022 ±.0069 to 0.029 ± 0.012 mmoles/mg protein. $p=0.07$).
Conclusion: Pro-inflammatory cytokines may induce changes in β-cell nutrient metabolism related to cell defence and repair, thus diverting essential energy production away from insulin secretion. These effects may be mediated by changes in expression and activity of key metabolic enzymes. However, modulation of insulin secretion after LPS exposure does not appear to be a result of changes in nutrient metabolism, suggesting that Toll-like receptor mediated signal transduction may impact on the insulin secretory process.
Support: HRB

PS 31
Mechanism of beta cell death

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Endoplasmic reticulum stress induced by thapsigargin activates both MAPK and NFκB pathways, whereas nitric oxide donor only activates MAPK in insulin-producing INS-1E cells

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Background and Aims: Pro-inflammatory cytokines, such as Interferon (IFN)-γ and Interleukin (IL)-1β trigger intracellular signalling through several different pathways leading to modified gene expression, cellular dysfunction and apoptosis in β-cells. Cytokine-induced expression of the gene inducible NO synthase (iNOS) leads to excessive nitric oxide (NO) production, which has several cytotoxic effects associated with cytokine-induced apoptosis in β-cells. NO depletes endoplasmatic reticulum (ER) Ca^{2+} stores and has been shown to be an important factor for cytokine-modified late gene expression. Accordingly, microarray analysis of cytokine-induced gene expression showed NO-dependent downregulation of the ER residing Sarcoplasmatic/ER Ca^{2+}-ATPase (SERCA)-2b. These events might disturb proper ER function and contribute to dysregulation of Ca^{2+} homeostasis leading to ER stress and apoptosis in cytokine-treated β-cells. The aim of the present work has been to address the potential role of ER stress on cytokine-induced β-cell apoptosis and to compare intracellular signalling in β-cells induced by ER stress and NO, using a SERCA2 blocker and a NO donor.
Materials and Methods: Rat insulin-secreting INS-1E cells were treated with cytokines (IL-1β and IFNγ), NO donor S-nitroso-N-acetyl-D,L-penicillamine (SNAP) and SERCA2 blocker Thapsigargin. Cell death was determined by ELISA detection of histone-DNA complexes in the cytoplasm. Activation of MAPKs was determined by Western blot analysis using phosphospecific antibodies and nuclear translocation of NFκB was determined by electric mobility shift assay (EMSA).
Results: We verified at the protein level that cytokine-induced suppression of SERCA2b expression is NO-dependent in insulin-producing INS-1E cells. The three subgroups of Mitogen-activated Protein Kinases (MAPK) ERK, p38 and JNK were activated by thapsigargin and SNAP, although with different kinetics. Furthermore, thapsigargin, but not SNAP induced degradation of the NFκB inhibitor IκB and binding of NFκB to DNA. Both thapsigargin and SNAP caused apoptosis as determined by Western blot analysis of caspase-3 and by detection of histone-DNA in the cytoplasm.
Conclusion: Our data suggest that cytokines, via NO production, lead to ER stress by downregulation of SERCA2b. Events downstream to ER stress include activation of MAPK and NFκB.
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Characterization of the endoplasmic reticulum stress response in beta-cells by microarray analysis using the ApoChip

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Background and aims: IL-1β + IFN-γ, via NO synthesis, decreases expression of the sarcoplasmic reticulum pump Ca2+ ATPase (SERCA2b). This depletes endoplasmic reticulum (ER) Ca2+ and activates an ER stress response, contributing to beta-cell apoptosis. In order to characterise the genes expressed in beta-cells during ER stress, we performed microarray analysis of INS-1E cells exposed to cyclopiazonic acid (CPA), a reversible blocker of the SERCA pump. Microarray analysis was performed using the ApoChip, an array designed and printed by our research groups. The ApoChip is a beta-cell apoptosis targeted oligonucleotide array containing around 600 rat genes. These genes were selected based on the information we obtained by several high density oligonucleotide array analysis (using the Affymetrix® system) of beta-cells exposed to different pro-apoptotic stimulus; it also contains others genes related to apoptosis and ER stress in other tissues.
Materials and methods: INS-1E cells were exposed for different time points to: 1. Medium alone; 2. The carrier solvent of CPA (DMSO; 0.04%); 3. CPA (12,5–25 μM). The cells were then harvested for RNA extraction (RT-PCR, microarray analysis) or luciferase assay. Activation of XBP1/ATF6 pathways by CPA was measured using a luciferase construct containing five ATF6/XBP1 binding sites. Microarray experiments were performed in

duplicates and each sample was hybridized on two different ApoChips. Results are given as mean±standard error.
Results: We confirmed that CPA triggers the ER stress response in INS-1E cells, as observed by activation of the 5xATF6/XBP1 reporter construct (3.9 ± 0.3 fold increase; n=5; p<0.001), IRE-1-mediated splicing of XBP1 and induction of CHOP mRNA expression (4.7 ± 0.8 fold increase; n=5; p<0.02). Continuous exposure of INS-1E cells to 25 µM CPA for 12–24 h induced respectively 21 ± 1% and 55 ± 1% apoptosis, as compared to 3.1 ± 0.3% apoptosis in control (n=6). Removal of CPA from the medium after a 12 h exposure allowed beta-cells to recover and survive, implying that these cells trigger effective defense mechanisms against ER stress. Microarray analysis of INS-1E cells exposed to CPA (25 µM) for 15 h showed an up-regulation of 24 genes and down-regulation of 33 genes (using a 2 fold variation as cut-off point). The biological variability between the two independent samples and average variability between the chips was in the range of 20–25%. We observed a decreased expression of genes related to beta-cell function, namely insulin and proinsulin convertase, while there was an upregulation of genes related to ER stress response/apoptosis including CHOP (6-fold increase, confirmed by RT-PCR), PERK (1.8-fold increase) and caspase 12 (2-fold increase).
Conclusions: 1.CPA induces an ER-stress response in beta-cells and leads to modification of key genes involved in beta-cell function and ER stress response/apoptosis; 2. Beta-cells have the capacity to recover from a severe ER stress; 3.Our "home made" microarray, the ApoChip, is a reliable tool to assess beta-cell gene expression in the context of apoptosis.
Support: JDRF Center for Prevention of Beta-cell Destruction in Europe; European Foundation for the Study of Diabetes (EFSD)-JDRF-NovoNordisk-Programme in Type 1 Diabetes; the Fonds National de la Recherche Scientifique (FNRS, Belgium) and Communauté Francaise de Belgique- Actions de Recherche Concertées (ARC)

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Expression of peroxiredoxins in rat pancreatic β-cells
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Background and Aim: The autoimmune destruction of the insulin-producing beta cells in type 1 diabetes mellitus (T1DM) is mediated in part by cytokines (IL-1β, IFN-γ, TNF-α). The cytokines are cytotoxic to beta cells by inducing the formation of oxygen free radicals, nitric oxide, and peroxynitrite. Pancreatic islets and some insulin-producing cells in culture are known for their low antioxidant enzymes expression and their susceptibility against reactive oxygen and nitrogen species. Peroxiredoxins (Prdxs), a family of peroxidases well conserved from bacteria to humans, are composed of six members in mammals. These antioxidant enzymes are known to reduce hydrogen peroxide, alkyl hydroperoxides and are also able to react with and detoxify peroxynitrite. We aimed to study the occurence of peroxiredoxins (Prdxs1-6) in pancreatic rat beta cells and to determine whether expression of these enzymes is regulated by cytokines.
Materials and Methods: The cell specific expression of Prdxs in adult rats pancreas was investigated by immunohistochemistry and the rat kidney was used as a positive control. Expression of these proteins in cultured INS-1E was assessed by immunofluorescence microscopy. After 24 hours cytokines treatment (IL-1β 10 U/ml, IFN-γ 100 U/ml) of the INS-1E cells, the viability was determined by the MTT assay. The dichlorodifluorescein (DCF)-sensitive intracellular ROS were measured by fluorescent spectrometry. The expression of Prdxs in INS-1E cells was investigated by Western blot analysis, MnSOD being used as a positive control and beta actin being the reference.
Results: All the 6 Prdxs were expressed in rat pancreatic islets. However, the distribution was very heterogeneous. The Prdxs1, 4, 5 appeared to be more expressed in the islets than in the exocrine tissue. The Prdx2 seemed to be highly expressed in non beta cells, while Prdx6 was poorly expresssed. The Prdx3 was ubiquitously found in the pancreas, probably located in mitochondria. In order to determine the pattern of expression of the Prxs in the insulin cell line INS-1E, the cells were submitted to immunocytochemistry with the six primary antibodies against the Prdxs1-6. The 6 Prdxs were expressed in the insulin cells, and the specific subcellular locations of Prdx5 and Prdx3 in mitochondria was confirmed. When the INS-1E cells were treated by cytokines for 24 hrs, we observed 50% reduction in the cell viability (p<0.01). Congruent with the cell viability assay, the ROS detection technique showed that the DCF fluorescence was three times higher in the group treated by cytokines compared to control (p<0.01). Treatment by aminoguanidine completely inhibited DCF fluorescence in the treated group, without changing the fluorescence in controls. Preliminary experiments in Western blotting suggested that Prdxs expression was not modulated by a stress induced by cytokines in INS-1E cell lines compared to control, although MnSOD expression was upregulated in these conditions.
Conclusion: Prdxs(1-6) were expressed in rat pancreas as well as in insulin cell line. The INS-1E responded significantly to the mixture of cytokines by decreasing cell viability, producing reactive oxygen species and overexpressing MnSOD. However after 24 hours cytokines treatment, we didn't observe any overexpression of Prdxs in the insulin secreting cell lines.

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Protein expression profiles in islets of Langerhans during allograft rejection and spontaneous diabetes development
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Background and Aims: Islet allograft transplantation manin has been used as a means to circumvent the need for insulin administration and has in some cases been able to restore endogenous insulin production. However, immunosuppression is required to prevent the graft from being rejected and destroyed. Changes in protein expression pattern during spontaneous diabetes development in the diabetes prone BioBreeding rat (BB-DP) have previously been reported. In the present study we have investigated if any of the changes in the protein expression pattern seen during diabetes development in syngeneic islet transplants are also present during allograft rejection.
Materials and Methods: Two hundred neonatal islets were syngeneically transplanted under the kidney capsule of 30 day old prediabetic BB-DP rats and removed at day 7 and 37 after transplantation (just before cellular infiltration) and at onset of diabetes (n=6 in all experiments). Two hundred neonatal BB-DP islets were allogeneically transplanted under the kidney capsule of 30 days old Wistar Kyoto (WK) rats (n=3 in all expriments) and removed before onset of allograft rejection (day 7) and day 12 after transplantation at maximal islet graft inflammation (rejection) and were labelled with [^{35}S]-Methionine. The protein expression profiles of all the transplants were visualised by 2 dimensional gel (2-DG) electrophoresis, analysed and compared.
Results: Three hundred and ten protein spots (of total 2588) had changed level of expression(p<0.01) in syngeneic transplants in the BB-DP islets from day 7 posttransplantation until day of diabetes onset. In BB-DP islets transplanted to WK rats 53 protein spots showed changes in expression level when comparing grafts removed day 7 with grafts removed on day 12 posttransplantation where mononuclear cell infiltration is at a maximum. Only 4 (1%) were significantly changed in both syngeneic (autoimmune) and allograft destrution. When protein expression changes from syngeneic transplants from day 37 posttransplantation to day of onset (maximal mononuclear graft invasion) were compared with protein expression changes in allografts from day 7 to day 12 only 3 spot expression changes were found in both situations.
Conclusion: Our data suggest that changes in islet protein expression seen during autoimmune (syngeneic) and allograft destruction of BB-DP islets are different. If this result reflects activation of distinct signalling pathways in islet cells is currently unknown.

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Vitamin E homologs protect cultured islets from anoxia-induced death: implications in islet processing and engraftment
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Background and Aims: Islet cell death induced by hypoxia or anoxia is a major factor limiting viable islet yield and engraftment, and ultimately success rate of islet transplantation. This work explores the effect of media supplementation with alpha- and gamma-tocopherol (forms of Vitamin E, known to have antioxidant as well as non-antioxidant cytoprotective properties) in protecting porcine and human islets from death induced by exposure to anoxia (95% N_2/5% CO_2).
Materials and Methods: Porcine and human islets were isolated and then cultured for 48 hours according to standard protocols. Islets were then cultured for an additional 18–24 hours in the presence or absence of alpha- and gamma-tocopherol (used in combination, 50 µM each). Islets from each group were then cultured in their respective media for an additional 24 hours exposed to a gas phase of either 95% air/5% CO_2 (control) or 95% N_2/5% CO_2 (anoxia). Islet quality was assessed at 6 and 24 hours after the initiation of exposure to anoxia by measuring Oxygen Consumption Rate (OCR), ATP, and Caspase 3/7 activity (all normalized to DNA), microphotography, and transplantation into diabetic nude mice. Data are reported as mean ± standard deviation for triplicate measurements.

Results: Viability of controls did not change significantly with time. OCR/DNA measurements demonstrated that exposure to anoxia for 6 hours was sufficient to reduce islet viability to 44 ± 13% of the respective control. Extending anoxic exposure to 24 hours further reduced viability to 35 ± 1% of its control and resulted in severe islet disintegration (single cells and small aggregates). Supplementation with alpha- and gamma-tocopherol maintained viability at 91 ± 9% and 72 ± 4% of their controls at 6 and 24 hours, respectively, and prevented islet disintegration. ATP and Caspase activity measurements indicated trends consistent with the OCR measurements. Loss of islet viability under anoxia as well as protection by supplementation was confirmed by transplantation in nude mice. Similar results were obtained in preliminary experiments with human islets.
Conclusions: A relatively inexpensive combination of natural compounds already approved for human use offers strong protection of islets from death induced by anoxia. Supplementation of preservation, isolation, and culture media, as well as transplant recipients with alpha- and gamma-tocopherol is expected to improve viable islet yield, promote engraftment, and markedly improve islet transplantation outcome.

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Survival of β-cells in human islets during oxygen deprivation is influenced by islet size
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Background and Aims: Clinical islet transplantation is limited by the high amount of islet tissue required to achieve insulin independence. Oxygen depletion in the liver mainly during the early post transplant period is a major factor contributing to primary non-function of the islets. The aim of the present study was to assess the contribution of isolation procedure and hypoxic culture conditions (simulating the oxygen supply in the portal system) on apoptosis/necrosis.
Materials and Methods: Human islets were cultivated under normoxic or hypoxic conditions (1% O_2) for 1 to 7 days. Islets were stained for active caspase-3 and insulin. Caspase-3 positive cells were counted and the insulin positive area was measured. Necrosis was assessed by H&E staining.
Results: Freshly isolated islets showed a higher number of caspase-3 positive cells per area-unit (2.0 ±SD 1.8 cpa) than islets cultivated under normoxic conditions for 1, 3 and 7 days (1.2 ± 0.9; 1.2 ± 1.4; 0.9 ± 1.2 cpa; $p<0.002$). In freshly isolated islets caspase-3 staining differed between small and large islets (1.9 ± 1.5 vs. 3.6 ± 2.9 cpa; n.s.). Under normoxic conditions the extent of necrosis/apoptosis did not differ. After 24 h of hypoxia, however, larger islets were more affected by central necrosis as compared to small once (even more pronounced after 48 h), whereas the number of caspase-3 positive cells did not change considerably.
Conclusion: In conclusion, under normoxic culture conditions pO_2 is sufficient for survival of even large islets, but as previously shown, at the expense of insulin production. In the portal circulation (hypoxic condition), however, the core of islets, consisting mainly of β-cells, is more vulnerable to necrosis. Although larger islets contribute significantly more to the islet equivalent number (IEN; i. e. high isolation index) as compared to smaller islets, they are at increased risk to be lost during or shortly after transplantation due to the greater vulnerability. Therefore, islet isolations with a smaller isolation index and smaller IEN might be preferable for transplantation due to their higher resistance to hypoxia.

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Mitochondrial peroxiredoxin III protects pancreatic beta cells against apoptosis
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Background and Aims: Type 1 diabetes mellitus is characterised by a progressive autoimmune destruction of insulin-producing beta cells in the pancreas. Macrophages and cytokines induce the synthesis of reactive oxygen and nitrogen species. Pancreatic islet cells are very sensitive against this attack due to its low content of well-known antioxidant enzymes like superoxid dismutase or catalase. Especially the resulting mitochondrial damages give an important signal to promote beta cell death. For this reason, there is considerable interest in peroxiredoxin III (Prx III, Mer5, SP-22, and AOP-1) and its function in pancreatic islets. Prx III is localised in mitochondria and belongs to a family of highly conserved thioredoxin-dependent peroxide reductases, the only inducible antioxidant enzymes which can protect against both, oxidative and nitrosative stress. The aim of our studies was to elucidate whether Prx III-thioredoxin-system can be applied as defence system to prevent beta cell destruction.
Materials and Methods: Using immunohistochemical, northern and western blot analyses, we determined the expression of Prx III in mouse pancreas and the rat insulinoma cell line RINm5F. To further examine the role of this antioxidant enzyme in beta cell protection, we established stably transfected RINm5F cells over-expressing or down-regulating Prx III after incubation with doxycyclin. These cells were treated with TNF α and streptozotocin and caspase activities were measured.
Results: The detection of Prx III in the pancreas revealed that this enzyme is predominantly expressed in the islet cells. An enhanced amount of mitochondrial Prx III in stably transfected cells protects the RINm5F cells against several stressors as revealed by measurement of caspase-3 and 9 activities. Down-regulation of this antioxidant enzyme resulted in increased caspase activities.
Conclusion: The results demonstrate that the activation of the mitochondrial Prx III-thioredoxin-system may help to protect beta cells against oxidative as well as nitrosative stress occurring by autoimmune attacks during pathogenesis of type I diabetes.

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Stable and efficient gene transfer into islets of Langerhans using an improved lentiviral vector system
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Background and Aims: Type 1 diabetes mellitus is characterized by an insulitis which leads to a selective destruction of pancreatic beta cells. Cytotoxic T-cells infiltrating islets of Langerhans release soluble mediators such as cytokines, oxygen free radicals, and nitric oxide. These mediators contribute directly or via different pathways to the beta cell death. The transfer of potentially cytoprotective genes is an interesting concept to analyse the signal pathways and to identify targets for intervention, through which the process of cell death could be blocked. However, efficient vector systems are essential for the gene transfer. The outstanding feature of a lentiviral vector system is the possibility of a stable transduction of primary mitotically inactive cells. But there are limitations for the infection of three-dimensional cell clusters like islets of Langerhans. Only the outer layer of cells and not the inner core can be transduced due to limited penetration of the viral particles. It was the aim of this study to establish an efficient technique for the transduction of whole islets of Langerhans by the combination VP22 fusion proteins with a lentiviral vector system. The Herpes simplex virus type 1 tegument protein VP22 is able to penetrate the membrane of adjacent cells as a fusion protein and to mediate an intercellular transport.
Materials and Methods: The VP22 cDNA was subcloned in frame with cDNA of the reporter genes EGFP and EYFP into the lenti plasmid pLenti6/V5-MCS. Lentiviral particles were generated by a standard protocol. The VP22-EGFP or VP22-EYFP lentiviruses were used to transduce RINm5F, MIN6, COS-1, MIN6 pseudo-islets and mouse islets with different multiplicity of infection. The expression level and the intercellular transport was analysed by fluorescent microscopy und immunostaining of VP22 protein and SV40 antigen.
Results: For both N- and C-terminal VP22-EGFP fusion proteins a homogenous cytosolic localisation was detectable after lentiviral transduction of RINm5F, MIN6 and COS-1 cells. The fluorescent intensity of the VP22-EGFP fusion proteins was 64% lower than in control cells transduced with EGFP. To prove the intercellular transport RINm5F cells expressing VP22-EGFP proteins after lentiviral transduction were co-cultivated with non infected MIN6 cells. After 3 days of co-cultivation an intercellular transport of VP22-EGFP fusion proteins from RINm5F cells to MIN6 cells could be verified by double immunostaining with specific monoclonal anti-VP22 and SV40 antigen antibodies. For the analysis of the spread of VP22 fusion proteins in three-dimensional cell cluster like islets of Langerhans MIN6 pseudoislets and mouse islets were lentivirally transduced with VP22-EGFP. By fluorescent microscopy it was possible to show that the outer layers of infected cells were able to release VP22-EGFP fusion proteins to the cells in the core of the islets.
Conclusion: This new transduction technique allows an efficient gene transfer into whole islets of Langerhans and opens new perspectives for the analysis of the signal pathways leading to beta cell death and the protection of islets against cytokine mediated cell death.
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PS 32
Beta cell degeneration

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Indoleamine 2, 3-dioxygenase induction by interferon gamma (IFNγ) in human and mouse islets

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Background and Aims: Indoleamine 2, 3-dioxygenase (IDO) catalyzes the initial, rate-limiting step of tryptophan catabolism along the kynurenine pathway and has been implicated in immunoregulatory responses to fetal tissue and intracellular parasites. Its role in cytokine responses in insulitis of type 1 diabetes was investigated in this study.
Materials and Methods: Human and mouse islets isolated by collagenase digestion were incubated in various cytokine combinations for 6–72 h and analyzed for IDO mRNA by microarray and RTPCR and enzyme activity by colorimetric assay and hplc.
Results: Massive increases in IDO mRNA (100–10000 fold) were observed in both human and C57B/6J mouse islets in response to interferon gamme (IFNγ) exposure for 24-h by microarray and quantitative-PCR. By contrast IL-1β, IL4, IL6 and TNF had little effect. The absolute level of IDO mRNA was consistent with its location in a major cell type and since the induction by IFNγ did not correlate with the levels of ductal tissue (CK19 and Trf 2) and acinar tissue (amylase and elastase) contamination we concluded that the pancreatic endocrine cells were the principal source of IDO. Increased kynurenine production was detected in the media of IFNγ-treated islets by colorimetric reaction (Ehrlich's reagent) and by hplc analysis. These changes correlated with increased IDO enzyme activity in homogenates of islets in response to IFNγ-treated human islet. The addition of 1 α-methyl tryptophan and IL 4 to the media inhibited the IDO enzyme response.
Conclusion: The kynurenine pathway may play a role in the response of islets to inflammatory stimuli and in the short term protect the islets by limiting the local availability of this essential amino acid to infiltrating lymphocytes and dendritic cells. Induction of IDO in the longer term however may be deleterious as tryptophan metabolites such as kynurenine, anthranilic and picolinic acid are themselves cytotoxic.

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Serum from prediabetic BB-rats affects pancreatic islet cell function and viability

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Background and aims: The BB-rat develops type 1 diabetes at the age of 50–70 days, which gives an excellent opportunity to study the impact of prediabetic conditions on disease onset and progression. Our aim was to investigate if sera from prediabetic BB-rats influence pancreatic islet cell function and viability.
Material and methods: Changes in cytoplasmic free calcium concentration ($[Ca^{2+}]_i$) were measured by microfluorometry and cell viability with a MTT-assay. Dynamics of insulin release, in response to glucose stimulation, were studied in islets from prediabetic and control rats incubated with their own sera for 24 h. Morphological comparisons were done on pancreatic sections stained with Hematoxylin/Eosin and van Gieson's or subjected to immunohistochemistry.
Results: Changes in $[Ca^{2+}]_i$ subsequent to depolarization with 25 mM K^+ were investigated in islet cells from control BB-rats that had been incubated for 24 h with 60-days old prediabetic or control sera. Of the 29 tested sera 19 (65%) induced a significantly higher increase in $[Ca^{2+}]_i$ compared to sera from age-matched control rats. The sera that had this effect on $[Ca^{2+}]_i$ are referred to as positive. When analyzing cell viability with a MTT-assay, a significantly lower viability was seen in cells exposed to 3/3 positive 60-days sera, but not in cells exposed to negative 60-days sera. Islets from 60-days old prediabetic rats had a lower insulin release, when stimulated with 11 mM glucose, compared to control rats. Islets in pancreatic sections from 60-days old prediabetic rats had a lower number of insulin-containing cells compared to age-matched controls. Statistical significance was evaluated by Student's t-test and p values < 0.05 were considered significant.
Conclusion: These results indicate that sera from prediabetic BB-rats can influence islet cell $[Ca^{2+}]_i$, insulin release and viability. Work in progress will identify both the responsible serum factor(s) and the extent to which intervention with it will delay onset of β-cell destruction and thereby development of diabetes in this animal model of type 1 diabetes.
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The niche hypothesis: non-β islet endocrine cells replicate to fill the void left by β-cell loss in diabetes

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Background and Aims: Autoimmune diabetes is characterized by a histopathological lesion of infiltrating T cells targeting islet β-cells. Non-β islet endocrine cells are spared this autoimmune destruction, resulting in a relative expansion of these cells within diabetic islets. To examine how changes in non-β islet endocrine cell populations develop during autoimmune diabetes, we examined pancreas from prediabetic and diabetic female NOD mice.
Materials and Methods: Pancreas was excised from 4–30 week old NOD mice and immunostained for insulin, glucagon, somatostatin, pancreatic polypeptide (PP), ghrelin, CD45 and Ki-67. α-, β- and δ-cell areas were compared in pancreas at 4 (non-diabetic; n=3), 12 (pre-diabetic; n=3) and 18–24 weeks (diabetic; n=5) of age.
Results: As expected, β-cell area decreased from 61.7 ± 0.5% of total islet area in the non-diabetic to 50.6 ± 8.5% in the pre-diabetic group, and was significantly decreased to 2.3 ± 1.5% in the diabetic group ($p<0.001$). This decrease in insulin-positive cells was accompanied by an increase in CD45-positive islet-infiltrating cells that peaked in the pre-diabetic group, at a time when the animals had not yet developed hyperglycemia. In contrast, the islet area comprised of glucagon- and somatostatin-positive cells increased significantly from 16.4 ± 0.2% and 6.9 ± 1.4% in the non-diabetic group to 35.2 ± 3.1% ($p<0.001$) and 24.2 ±%2.1 ($p<0.01$) in the diabetic group, respectively. In the pre-diabetic group, although the proportion of islet area comprised of glucagon- (11.9 ± 2.8%) and somatostatin-positive (5.4 ± 1.3%) cells was not significantly different from the non-diabetic group an increase in the number of glucagon- and somatostatin-positive cells was apparent in those islets with a clear loss of β-cells. This finding suggests that hyperglycemia *per se* does not drive non-β cell expansion in diabetes. Once the insulitic lesion dissipated and the animals were overtly diabetic, the islets were almost entirely comprised of α- and δ-cells. Double-staining with Ki-67 revealed, in addition to a marked α- and δ-cell proliferation, an increased proliferation of PP-cells in the head of the pancreas at 12–14 weeks of age, indicating that all non-β endocrine cells partake in islet remodeling following β-cell destruction. No increase in ghrelin-positive cells was observed in pre-diabetic or diabetic NOD islets, making it unlikely that an expansion of this putative β-cell progenitor occurs in an effort to replace loss of β-cells in diabetes.
To determine whether loss of β-cells is sufficient for expansion of non-β islet cells or whether infiltrating immune cells are required, we induced diabetes in female Balb/c mice with streptozotocin (STZ, 275 mg/kg). In two groups of STZ-diabetic mice, we replaced insulin and normalized blood glucose by insulin implant or subrenal transplant of 300 syngeneic islets. Replication of α- and δ-, and PP-positive islet cells occurred in STZ-diabetic mice regardless of whether insulin was replaced or not. These data indicate that neither islet inflammation nor prolonged hyperglycemia or hypoinsulinemia drives the endocrine cell expansion following β-cell destruction.
Conclusion: Proliferation of α-, δ- and PP-cells following loss of β-cells in animal models of type 1 diabetes does not appear to be driven by insulitis, hyperglycemia or hypoinsulinemia. We propose that replication of non-β islet endocrine cells occurs to fill the void left by loss of β-cells within the diabetic islet.

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Pancreatic β-cell apoptosis is induced by amylin fibril formation through inhibition of ubiquitin-proteasome pathway

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Background and Aims: Amylin aggregation has a critical role in the development of islet amyloidosis in patients with type-2 diabetes. It is known that islet amyloid deposits are toxic to pancreatic β-cells and related to undergo apoptosis. However, the molecular mechanism by which islet amyloid mediate cytotoxicity remains poorly understood. The aim of the present study was to investigate *in vitro* the molecular mechanism of human amylin cytotoxicity in the promotion of pancreatic β-cell apoptosis.
Materials and Methods: *Cell culture and treatment:* Mouse pancreatic β-cell line MIN6 was cultured in DMEM, followed by treatment for 2, 12 or 24 h with either 1, 10 or 20 μM human amylin. *Apoptosis assays:* FACS analysis of doubled stained cells with annexin V-FITC and 7-AAD. Western-blot evaluation of activated caspase 3 fraction. *Morphologycal analyses:* Congo red staining and transmission electron microscopy. *Genome expression profile:* RNA isolation, Affymetrix oligonucleotide microarray hybridization, bioinformatic analyses and real-time quantitative RT-PCR. *Proteasome activity assays:* Either pZsProSensor-1 or pUb^{G76V}-EGFP was transient transfected into MIN6 cells before human or rat amylin treatment. Cells were also cultured with proteasome inhibitor ALLN and proteasome activator PA28.
Results: MIN6 cells cultured at different concentrations of human amylin showed ultrastructural changes evidenced by plasma membrane alterations. Gene expression profile of the same cultures revealed overexpression of transcripts that codified for several heat shock proteins (Hsps): heat shock protein-1-alpha, heat shock protein-1-beta and tumor rejection antigen-gp96. These observations confirmed that cellular stress due to amyloidogenic membrane disruption resulted in dysregulation of the chaperone pathway. When proteasome activity was impaired in MIN6 cells by the presence of proteasome inhibitor, up-regulation of *Hsp*s was also detected ($p<0.05$). Furthermore, quantification of proteasome activity of cultures with different human amylin administrations showed a reduction in proteasome function (>100% at 24 h; $p<0.001$) that contributed to intracellular accumulation of ubiquitinated proteins (>100% at 24 h; $p<0.001$) leading to a functional suppression of the ubiquitin-proteasome pathway. No changes in ubiquitin-proteasome pathway were observed with equivalent rat amylin treatment. Although the presence of amyloidosis, there was no apoptosis when proteasome activator PA28 was added in MIN6 cells.
Conclusion: Functional inhibition of ubiquitin-proteasome pathway is the cytotoxic mechanism by which amylin fibril deposition induced apoptosis in pancreatic β-cell.
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Activation of ATF-2 by p38 MAP kinase during apoptosis induced by human amylin in cultured pancreatic beta-cells

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Background and Aims: Current studies have indicated that amyloid formation may contribute to the development of hyperglycemia by causing islet dysfunction. Fibrillogenic amylin has been shown to evoke β-cell apoptosis through linked activation of a caspase cascade and JNK 1. The aim of this study was to determine whether the other two MAP kinases, ERK and p38 kinase, can affect this apoptotic pathway and, if they do how they might cooperate to activate their downstream target genes, such as ATF-2.
Materials and Methods: Two insulinoma cell lines. Rat RINm5F and human CM cells, were cultured and exposed to human or rat amylin. Activities of p38 and ERK were measured by western blot and immunocomplex kinase assay. The role of activation of ATF-2 in apoptosis was determined by cell death ELISA using cells pretreated with selective kinase inhibitors. Changes of activated ATF-2 in *CRE*-DNA binding activity and *CRE*-mediated transcriptional activity were analyzed by electrophoretic mobility shift assay (EMSA) and reporter gene assay, respectively.
Results: p38 kinase was activated, whereas ERK was unaffected, by human amylin, but not by non-fibrillogenic rat amylin, in both RINm5F and CM cells. Pre-treatments with SB203580 (a p38 kinase inhibitor) alone, with SB203580 and JNK inhibitor I or with the combination of SB203580, JNK inhibitor I and a caspase-8 inhibitor, decreased human amylin-induced apoptosis and caspase-3 activation by about 30%, 70% and 100%, respectively. Human amylin induces time-dependent activation of ATF-2, which can be largely suppressed by SB203580. In addition, EMSA detected increased *CRE* DNA binding activity. Reporter gene assays detected increased *CRE*-mediated transcriptional activity and *c-jun* promoter activation.
Conclusion: This study has demonstrated increased p38 kinase activity, but not ERK activity, in human amylin-induced β-cell apoptosis. Activation of multiple pathways, including those mediated via JNK, p38 and caspase-8, are required. It was further shown that ATF-2 is the major downstream target of p38 kinase. Increased phospho-ATF-2 was found to be associated with increased *CRE* DNA binding activity and *CRE*-mediated transcriptional activity, as well as enhancement of *c-jun* promoter activation. We also detected changes in the phosphorylation status and composition of the CRE complex that may play important roles in regulation of the distinct downstream target genes. These studies establish p38 MAP kinase-mediated activation of ATF-2 as an important mechanism of hA-evoked β-cell death which may serve as a target for pharmaceutical intervention and effective suppression of β-cell failure in type-2 diabetes.
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GLP-1 prevents dexamethasone-induced apoptosis in insulin secreting cells

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Background and aims: Glucocorticoid therapy is known to affect glucose homeostasis which may escalate into diabetes mellitus. This implicates a dysfunction of beta cells. The inhibition of insulin secretion after glucocorticoid treatment has been described previously. Effects of glucocorticoids on beta cell mass and cell survival, however, is less well studied. The aim of the present study was to examine effects of the synthetic glucocorticoid dexamethasone (dex) on beta cell survival.
Materials and methods: INS-1 cells were pretreated in culture with 100 nM dexamethasone (dex) or vehicle for 1 to 4 days in the presence of test substances. Proliferation was examined after cell culture for 24 h in serum free medium containing 5 mM glucose by incorporation of BrdU (bromodeoxyuridine labelling for 8 h). Cell death was quantified by the percentage of DAPI-stained condensed nuclei and caspase-3 activity. Changes in transcription were estimated by semiquantitative real time PCR.
Results: Proliferation induced by glucose (16.7 mM) or IGF-1 (10 nM) was inhibited by dex. Pretreatment of INS-1 cells with dex time-dependently augmented INS-1 cell death. Condensed nuclei increased from 4.7 ± 0.2% (1 day treatment) to 13.9 ± 2.4% (4 days treatment, n = 5) while under control conditions their percentage remained below 2.8%. Caspase-3 activity was increased in parallel. These effects of dex were completely reversed by the glucocorticoid receptor antagonist RU486, 1 μM. Exendin-4, a GLP-1 receptor agonist, 10 nM, reversed dex-induced cell death by 68%. This effect was not mimicked by 8CPT-Me-cAMP, 50 μM, a specific activator of EPAC II, but was antagonized by H89, 10 μM, an inhibitor of protein kinase A (PKA). The amount of mRNA of Bcl-2, an antiapoptotic factor, was decreased by 50%.
Conclusions: We propose that glucocorticoids reduce beta cell mass by inhibition of proliferation and activation of apoptotic pathways involving caspase-3. The stimulation of GLP-1 receptors overcomes dex-induced cell death via activation of PKA but not EPAC II.

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Gelsolin and beta cell survival

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Background and Aims: Understanding beta cell apoptosis and developing means to block it have assumed great importance as such apoptosis may play a major role in the pathophysiology of type 2 diabetes. The Ca^{2+}-dependent actin severing protein gelsolin has been shown to be either pro-

or anti-apoptotic in other cell types and is expressed in insulin-secreting cells. The aim of this study was to investigate the role of gelsolin in beta cell apoptosis.
Materials and Methods: Studies were performed on B1 and C3 MIN6 (transformed mouse beta cell) sublines. Apoptosis was induced by incubating cells for 48 h in low glucose/serum medium containing 5 mM glucose (vs. 25 mM control) and 1% foetal calf serum (vs. 15% control). Apoptosis was measured by ELISA or TUNEL and active (cleaved) caspase-3 by immunofluorescence and Western blot. The plasmid pcDNA3.1-HA-gelsolin was used for transient transfection to over-express the fusion protein HA-gelsolin. One day after transfection, apoptosis was induced by low glucose/serum (48 h). Transfected cells were identified by immunolabelling using anti-HA-tag and apoptosis (TUNEL-positivity) or caspase-3 activation (immunofluorescence) scored in transfected vs. non-transfected cells in the same dish. Results are mean±SEM (unless mentioned otherwise) for n independent experiments with levels of significance assessed by Student's unpaired t-test.
Results: It has been shown previously that gelsolin is expressed >100-fold higher in B1 than C3 cells, with lower levels of apoptosis. We now show in control conditions that the increased apoptosis in C3 cells is accompanied by a higher percentage of cells positive for active caspase-3 when compared to B1 cells (0.9 ± 0.2% and 0.26 ± 0.1% respectively, n=4, $p<0.022$). This percentage was increased in both C3 and B1 cells after induction of apoptosis by low glucose/serum (5 ± 1% and 2 ± 0.3% respectively, n=4, $p<0.05$). These results were confirmed by Western blot for cleaved caspase-3. To investigate whether differential expression of gelsolin might underlie these differences in apoptosis and caspase-3 activation, this protein was over-expressed by transient transfection of B1 and C3 cells. After induction of apoptosis by low glucose/serum, the percentage of TUNEL-positive cells was decreased by 74 ± 9% in B1 cells over-expressing HA-gelsolin vs. non-transfected cells (n=3, $p<0.002$) and by 87 ± 3% in C3 cells over-expressing HA-gelsolin vs. non-transfected cells (n=3, $p<0.001$). Over-expression of gelsolin thus abolished the normally increased apoptosis in C3 vs. B1 cells. Levels of active caspase-3, following induction of apoptosis, were also decreased by over-expression of gelsolin in both B1 and C3 cells by 83 ± 11% and 80 ± 4%, respectively (SD: n=2).
Conclusion: Levels of expression of gelsolin correlate with apoptosis in MIN6 cells with this protein apparently playing an important role in protecting beta cells against caspase-3-mediated apoptosis. Given the known function of gelsolin as an actin-severing protein in other cell types, it is postulated that actin-remodelling may underlie its anti-apoptotic role in beta cells.

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Targeted local activation of Fas in pancreatic beta-cells for treatment of insulinomas and disorders of pathological autonomic insulin secretion
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Background and Aims: The prognosis of patients with insulinomas is poor when multiple or malignant tumors are present. Also, pancreatectomy is frequently necessary to rescue patients from persistent hyperinsulinemic hypoglycaemia of infancy (PHHI, nesidioblastosis). Therapeutic compounds that selectively destroy autonomically insulin producing cells may cure both insulinomas and nesidioblastosis. The aim of this study is to target the apoptosis-inducing actions of Fas-ligand (FasL) specifically to autonomic pancreatic beta cells.
Methods and Results: We demonstrate that artificial immobilisation of the extracellular inactive domain of FasL on the cell surface by fusion to an appropriate immobilisation domain is sufficient to convert these fusion proteins to bioactive apoptosis inducing compounds. To target this principle to pancreatic beta cells, fusion proteins of FasL with a peptide domain of the glucagon-like peptide 1 (GLP-1) analog exendin 4 (FasL-Ex4) were generated. Receptors for the incretin hormone GLP-1 (GLP-1R) are specifically expressed on the surface of insulin producing cells and exendin 4 (Ex4) binds to GLP-1R with high affinity. Expression of GLP-1R was verified by RT-PCR, fluorescence immunocytochemistry and FACS phenotyping in pancreatic beta cell lines INS-1, betaTC3 and BHK cells stably over-expressing rat GLP-1R and in primary human pancreatic beta cells. Surface immobilisation of FasL-Ex4 fusion proteins specifically in GLP-1R positive cells was demonstrated by fluorescence immunocytochemistry and FACS. FasL dependent induction of apoptosis and activation of apoptotic signal transduction pathways in beta cells was further demonstrated.
Conclusion: These results demonstrate that FasL-Ex4 fusion proteins hold the potential as therapeutic compounds to target FasL apoptotic activity specifically to GLP-1R expressing insulin producing cells for non surgical treatment of insulinomas and nesidioblastosis *in vivo*.
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Islet and pancreas transplantation

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Human fetal islet transplantation in type 1 diabetics: comparison of immunological effects between single and multiple implantation regimes

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Background and Aims: Previous studies have suggested that the multiple transplantation (Tx) might be equally metabolically efficient to a single regime in human adult islet Tx. The aim of this study was to compare immunological parameters after each of the two regimens of human fetal islets (HFI) and to investigate eventually connection with metabolic parameter.
Materials and Methods: In group A (single Tx, 9 patients), 180+/–20 × 1000 HFI equivalentes (IEQs) were implanted in a single injection I.M. In group B (multiple Tx, 8 patients) islets were implanted in 3 consecutive injections (60+/–10 × 1000 IEQs) at 7 days intervals. We analyzed the following immunological parameters: CD_4/CD_8 T lymphocyte ratio (1); islet cell antibodies (ICAs) (2) and insulin antibodies (IAs) (3). As the metabolic parameter insulin secreting capacity (ISC) was estimated.
Results: We found that CD_4^+/CD_8^+ T cell ratio increased, peaked on day 90, similarly in both groups: (day -1: A:1.18+/-0.03; B:1.19+/-0.04; day 90: A:1.79+/-0.09, B:1.75+/-0.08, p=NS) immediately before the decrease in C-peptide levels and than rapidly decreased without statistical differences. The levels of ICAs did not change; the levels of IAs were increased before Tx and than decreased without statistical differences between the groups. The values of ICS increased after Tx and than decreased similarly as T cell ratio.
Conclusion: Our results demonstrated that multiple and single HFIs Tx regimen did not differ in the kinetics of immunological response presumably involved in the islet graft destruction. CD_4/CD_8 ratio increased as the C-peptide level decreased peaked on day 90 when the decrease of C-peptide started. These results may influence the clinical outcome of HFIs Tx in type 1 diabetics.

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Expression and hypoxic regulation of the endothelin system in endocrine cells of pancreatic islets

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Background and Aims: Success of pancreatic islet transplantation depends largely on sufficient blood supply until engraftment. Improved perfusion in whole organ transplantation has been achieved by antagonizing the vasoactive peptide endothelin-1 (ET-1). With regard to a prophylactic ET-1 antagonist treatment in islet transplantation, we studied the expression of endothelin and its receptors in pancreatic islets also under hypoxic conditions, reflecting post-transplantation ischemia.
Materials and Methods: Islets were analysed immunohistochemically for expression of the endothelin system. The presence of ET-receptors was assessed by ligand binding studies and the ability of ET-1 to stimulate phosphorylation of mitogen-activated protein (MAP) kinase. Insulinoma cell lines and islets were analysed for mRNA expression of ET-1, ET_A- and ET_B-receptors and their regulation by hypoxia by real-time PCR.
Results: ET-1, ET_A- and ET_B-receptor immunoreactivity was identified in endocrine cells of human and rat pancreatic islets. The corresponding mRNA was detectable in rat beta-cell lines and isolated rat and human pancreatic islets. Competition binding studies on rat islets revealed binding sites for both receptor types. ET-1 stimulated phosphorylation of MAP-kinase, which was prevented by ET_A- and ET_B-receptor antagonists. After exposure to hypoxia which simulates post-transplantation ischemia, mRNA levels of ET-1 and ET_B-receptor of human islets were robustly induced whereas ETA-receptor mRNA was slightly reduced.
Conclusion: These data demonstrate that the endothelin system is expressed in endocrine cells of pancreatic islets and that hypoxia affects the expression of all three components. In islet transplantation, careful evaluation of ET-1 antagonist treatment with respect to islet biology might be of clinical importance.

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Transplanted islet oxygenation before revascularization: the critical role of blood vessel density and proximity

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Background and Aims: In the posttransplant period before islet revascularization, islet cells depend on diffusion from the nearest blood vessels (BVs) for oxygen and nutrients. Large oxygen partial pressure (pO_2) gradients develop, resulting in anoxia that may limit islet viability and engraftment. Layers of dense islet tissue, as those implanted under the kidney capsule, are most susceptible to development of anoxia, but even single islets can develop anoxic cores if large or if ambient pO_2 is low, as in the liver. In this work we focus on the effect of BV density and proximity on oxygenation of a confluent islet monolayer in ranges centered around experimental observations available in the literature.
Materials and Methods: We have modeled with finite elements oxygen diffusion from a plane of parallel and uniformly spaced BVs to a planar slab of dense islet tissue with volume one-half that of a confluent square array monolayer of 150 μm diameter islets. As a worst case scenario, a uniform pO_2 of 40 mm Hg, corresponding to venous levels, was assumed at the surface of the BVs. Under this assumption blood flow rate is irrelevant and islet oxygenation is only a weak function of BV diameter. Reported results were derived with a uniform BV diameter of 10 μm. The same uniform pO_2 was assumed to be maintained by remote BVs at a distance of 100 μm from the islet layer surface. It was assumed that host tissue has a 10% cellularity and that islets are initially fully viable, consuming oxygen following Michaelis-Menten kinetics down to a critical pO_2 of 0.1 mm Hg. Simulation results are expressed as the fraction of islet tissue exposed to $pO_2 > 0.1$ mm Hg. In terms of this outcome, the relative error associated with the representation of the islets as a planar slab is less than 10%. We ran simulations for distances x between the centers of neighboring BVs ranging from 11 to 300 μm, and for distances y between the islet layer surface and the BV center-plane ranging from 5 to 95 μm.
Results: The assumption of a uniform pO_2 at $y = 100$ μm guarantees a minimum level of oxygenation close to 30%. 100% oxygenation was possible only for $x < 35$ μm (~30 BVs per mm of interface length) and $y = 5$ μm (BVs adjacent to the islet layer surface). For $x = 150$ μm, observed within 100 μm from a planar membrane diffusion device implanted subcutaneously in rats, predicted oxygenation is about 35–45%, while for $x = 70$ μm, observed in conjunction with local infusion of VEGF in the same device, predicted oxygenation is about 40–60%. BV densities within 15 μm from a vascularization-promoting membrane implanted subcutaneously in rats (x = 270 μm at day 7 and $x = 125$ μm at day 21 post-implantation) correspond to estimated oxygenation levels of 45–65%, assuming the same BV densities occur around a naked islet layer.
Conclusion: Theoretical predictions suggest that oxygenation of a confluent monolayer of transplanted islets before revascularization is limited to 35–60% if ambient pO_2 is close to venous levels, and that BV density and proximity play a critical role. If islets are stacked in a confluent bilayer, these fractions would be cut in half and anoxic cores could develop even at arterial ambient pO_2. Ways to improve oxygenation and transplantation outcome include increasing islet spacing and vascularization of the transplantation site prior to islet transplantation by local infusion of angiogenic factors.

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Health status of persons with diabetes mellitus type 1 after one-time pancreas and kidney transplantation

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Background and Aims: Assessment of the health status in the form of a) system review and b) studies of the pancreas and kidney function after elapsing 1–33 years sins the transplantation took place. Creates the possibility of verifying the adequacy of patient selection, performance of operation techniques and of immunological therapy after transplantation.
Materials and Methods: In the group of 19 of diabetes type 1 – 13 females and 6 males, aged between 25 and 54 years, with diabetes mellitus duration before one-time pancreas – kidney transplantation 16 – 33 years and after transplant 1,0 to 11 years – the complex clinical studies were performed. Patients were hospitalized and underwent: 1) organ status review (eyes, heart and vessels, nervous system) 2) assessment of transplanted pancreas

function – basal and reactive insulin, C-peptide and transplanted kidney function – creatinine clearance, albuminuria, CT 3) determination of selected metabolic indices - glycemia profile, HbA1C, lipidemia, urikemia, HOMA, 4) examination of psychosocial status.
Results: Eyes, heart, vessels and nervous system status as related to diabetes mellitus was comparable before and after transplantation – no progression signs were found. Basal and reactive (after glucose) insulin and C-peptide serum levels were decreased in 2, normal in 6 and elevated in 10 cases. The beta cells function correlated with HOMA. In 17 out of 19 persons normal glycemia and HbA1C no symptomatic diabetes mellitus was found; however in 11 cases mixed hiperlipidemia and elevated CRP were present. In 16 cases the transplanted kidney function was sufficient (GFR > 50 ml/min). The psychosocial status of all transplanted persons strikingly improved.
Conclusion: In long-time observational studies of diabetic type 1 persons after long time one-time pancreas and kidney transplantation and on long-time immunosupressive therapy the high efficacy of this procedure was stated. Systemic organ review, transplanted pancreas and kidney function, metabolic indices and psychosocial status in 17 out of 19 diabetics were very satisfactory, they did not show indices signs of diabetes mellitus. Such transplantation should be applied more often and more early – before hemodialysis takes place. It is also less costful, than chronic dialysis.

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Insulin release and insulin pulsatility in patients successfully transplanted with kidney-pancreas grafts

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Background and Aims: Patients with brittle type 1 diabetes and renal failure often undergo simultaneous transplantation with kidney and pancreas. Maintenance of normoglycaemia relies on adequate insulin release in response to variations in systemic glucose concentrations. In addition, insulin is released in pulses between meals. These pulsatile bursts give rise to oscillations in peripheral plasma insulin concentrations every 5–15 min, and probably represents more than 70% of the total insulin released from the pancreas. Changes in these pulsatile bursts (frequency and regularity) have been considered as early signs of failing glucose control and development of diabetes. The present study was undertaken to see if insulin pulsatility is fully preserved in patients with functioning pancreas grafts.
Material and Methods: Nine persons with functioning kidney-pancreas grafts (transplanted 10.4 ± 1.2 years ago, mean±SD) were compared with 10 healthy control persons matched for age, gender and BMI. Besides an oral glucose tolerance test (OGTT), an insulin pulsatility test was performed over 180 min. Blood samples for insulin and C-peptide measurements were drawn every minute for 2 hrs, and during the second hour insulin release was entrained by 1 minute glucose infusions every 10 min. During the third hour blood samples were drawn every 3rd minute, and insulin release was additionally stimulated by high dose glucose and arginine infusions, respectively.
Results: Fasting plasma glucose was not significantly different in the transplanted and the control group (5.3 ± 0.4 vs. 5.3 ± 0.2 mmol/l, mean±SD). Plasma creatinine (98 ± 8 vs 70 ± 3 µmol/l), insulin (90 ± 18 vs. 26 ± 4 pmol/l) and C-peptide (1004 ± 114 vs. 637 ± 62 pmol/l) were all higher in the transplanted group ($p \leq 0.01$ for all). Glucose excursion during the OGTT was similar in the two groups, but the transplanted patients had higher insulin and C-peptide concentrations at all time-points. Autocorrelation analysis showed that the frequency of insulin pulses was not significantly different at baseline (7.8 ± 0.4 vs. 9.2 ± 0.9, p=0.08), and evened out during entrainment (10 ± 0 vs. 10 ± 0). Similarly, spectral analysis showed that regularity measurements at baseline were slightly lower in the transplanted patients vs. the control group (8.0 ± 1.0 vs. 10.3 ± 0.8, p=0.07), but regularity increased in both groups during glucose entrainment (16.4 ± 1.4 vs. 12.3 ± 1.7, $p > 0.1$). Both groups responded in a similar way during high dose glucose and arginine stimulation.
Conclusion: Insulin pulsatility is well preserved in patients with functioning pancreas grafts. Both frequency and regularity of insulin pulses are almost similar to normal control persons in the basal state and during glucose entrainment. The patients have elevated C-peptide and insulin levels, which can be explained by insulin resistance and lack of first pass uptake of insulin in the liver.
Support: Novartis

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Hyperinsulinemia after pancreas-kidney transplantation

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Background and Aims: Simultaneous pancreas-kidney transplantation (SPK) has become the therapeutic option for a patient with type-1 diabetes mellitus and end-stage diabetic nephropathy. After transplantation, endogenous insulin is determined by the capacity of the transplanted pancreas and the prednisone immunosupression. The aim of our study was to determine whether the technique of pancreas venous drainage in SPK changes the circulating insulin levels.
Materials and Methods: In this open, prospective, parallel-group study, we included 24 SPK recipients (age 29–42 yr) with systemic venous drainage (SPK-S, n=12) and portal venous drainage (SPK-P, n=12). All recipients underwent a 75-g OGTT twice: between 3–6 mo and 12–24 mo post transplantation for the assessment of insulin and C-peptide secretion. Following transplantation all SPK recipients had been under identical immunosuppressive treatment.
Results: Following transplantation, all patients were normoglycemic during the whole period of 24 months, had normal HbA1c.
SPK recipients in both groups had higher basal plasma C-peptide levels when compared with controls age-matched ($P < 0.05$). Fasting hyperinsulinemia and significantly higher stimulated insulin secretion was observed only in SPK with systemic venous drainage. Table: Hyperinsulinemia in SPK recipients.
Conclusion: SPK recipients with systemic venous drainage have peripheral fasting and stimulated hyperinsulinemia. These study strongly suggest that in SPK-S patients hyperinsulinemia could have an impact on graft deterioration and the development of cardiovascular disease, and further work is needed to ascertain long-term consequences of the technique of pancreas venous drainage.

Hyperinsulinemia in SPK recipients

Group	SPK-S	SPK-S	SPK-P	SPK-P
Time after SPK (months)	6–12	12–24	6–12	12–24
Insulin (microU/ml)	12.8 (5.3)*	18.1 (8.4)*	5.0 (1.8)	6.7 (1.3)
C-peptide/insulin ratio	11 (2.2)*	12.1 (2.9)*	29.2 (12)	34.3 (11)
Total AUC insulin	4156 (528)*	4709 (480)*	1523 (320)	2190 (280)

results are means (±SD)
* $P < 0.05$, compared with SPK-P

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Higher plasma amylin and impaired beta cell function in type 1 diabetic patients after combined kidney pancreas transplantation

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Background and Aims: After successful simultaneous kidney pancreas transplantation (SPK) in type 1 diabetic patients (T1DM), glycemia may revert to a non-diabetic metabolism without exogenous insulin therapy. In response to hyperglycemia, beta cells release both insulin and IAPP (amylin), being also the latter involved in glucose homeostasis by reducing hepatic glucose production and delaying gastric emptying. Aim of this study was to investigate amylin kinetics, beta cell function (BF) and insulin sensitivity in T1DM with SPK during an oral glucose tolerance test (oGTT).
Materials and Methods: Plasma concentrations of glucose, free fatty acids (FFA) and hormones (amylin, insulin, C-peptide) were assessed before and during a 3 hour 75g-oGTT in 11 T1DM patients after successful SPK (25 ± 1 kg/m², 47 ± 2 yrs, 4f/7 m), thus reversed to nondiabetic state (basal glucose 95 ± 3 mg/dl) and in 9 matching nondiabetic controls (CNT) (24 ± 1 kg/m², 47 ± 2 yrs, 4f/5 m, 92 ± 3 mg/dl). Mathematical models for data analysis yielded: amylin secretion and clearance, insulin sensitivity

and secretion. BF was assessed with the adaptation index (insulin sensitivity × C-peptide secretion).
Results: Fasting concentrations of amylin, insulin and C-peptide were markedly higher in SPK (*amylin*: 19 ± 3 vs. 7 ± 1 pM of CNT; *insulin*: 20 ± 2 vs. 10 ± 1 µU/ml; *C-peptide* 2.5 ± 0.2 vs. 1.7 ± 0.3 ng/ml, each $p<0.015$), while that of glucose and FFA were similar. During oGTT, plasma amylin concentrations were ~2fold higher in SPK (*area under curve* 7218 ± 1094 vs. 3605 ± 524 pM min of CNT; $p=0.006$); since *clearances* were similar (0.034 ± 0.001 vs. 0.034 ± 0.003 min^{-1}), higher amylin is due to increased secretion. Insulin sensitivity (SPK: 412 ± 13 vs. 450 ± 20 ml min^{-1} m^{-2}) and FFA kinetics were similar while BF was reduced (34 ± 2 vs. 48 ± 4 nmol m^{-2}, $p=0.005$). A significant inverse correlation ($r=-0.45$, $p<0.05$) was found between BF and amylin levels.
Conclusion: T1DM after successful SPK exhibit higher fasting and post-glucose-load amylin concentrations. This reflects elevated secretion, since no change in clearance is observed. Beta cell insulin response to glucose challenge is also impaired in transplanted T1DM and appears to be related to the elevated amylin levels. Thus, this study (which for the first time evaluated amylin in SPK) seems to indicate that plasma amylin could be a clinically relevant parameter reflecting beta cell function of pancreatic grafts in T1DM.

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Metabolic effect of sirolimus versus mycophenolate mofetil on pancreatic graft function
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Background and Aims: Metabolic effects of immunosuppressive agents are of great importance in pancreas or islet transplantation. The aim of our study was to compare the glucose metabolism in Type 1 diabetic recipients of kidney and pancreatic grafts on tacrolimus-based immunosuppression in conjunction with sirolimus (RAPA) versus mycophenolate mofetil (MMF).
Materials and Methods: We examined 30 insulin-independent patients after simultaneous pancreas and kidney transplantation with systemic venous drainage of pancreatic graft (mean post-transplant period 15.2 ± 4.26 [SD] days). All recipients had a good function of the kidney graft (mean serum creatinine level 113.8 ± 49.7 µmol/L). Fasting glycemia, insulin levels, glycosylated hemoglobin (HbA_{1c}), standard intravenous glucose tolerance test (IVGTT) with coefficient of glucose assimilation (K_G) calculation and trough RAPA levels were assessed in pancreas recipients before elective steroid withdrawal (mean dose 12.7 ± 3.41 mg/day). Insulin sensitivity was evaluated by using the homeostasis model assessment (HOMA-IR). Total C-peptide and insulin secretions were calculated as areas under the curves from the serum levels during the IVGTT.
Results: The groups (RAPA vs. MMF) did not differ in age, BMI, post-transplant period, steroids daily dose, HbA_{1c} and fasting glycemia. We did not find any significant difference in response of IVGTT (K_G: 1.08 ± 0.48 vs. 1.09 ± 0.32%/min.). In RAPA group (n = 15) 5 patients had an abnormal response to glucose stimulus ($K_G < 0.8$%/min.), 5 ones had an impaired glucose tolerance ($0.8 \leq K_G < 1.2$%/min.) and 5 ones had a normal glucose tolerance ($K_G \leq 1.2$%/min.). In MMF group (n = 15) the abnormal response was present in 4 recipients, the impaired glucose tolerance in 6 ones and the normal glucose tolerance in 5 ones. Total insulin secretion during IVGTT and HOMA-IR were significantly lower in RAPA group (1826 ± 942 vs. 2599 ± 869 mIU/L, $p = 0.03$; 2.69 ± 1.73 vs. 4.41 ± 2.61, $p = 0.04$, respectively). Trough levels of RAPA (5.8 ± 2.8 ng/mL) had no significant impact on any of examined parameters.
Conclusion: Glucose tolerance measured with the use of IVGTT was similar in patients treated with sirolimus and mycophenolate mofetil. However recipients on sirolimus had significantly lower insulinemia during the test and consequently more favourable indices of insulin action assessed by the HOMA-IR.
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Partial graft dysfunction after pancreas transplantation is associated with reduced insulin secretion
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Background and Aims: Successful pancreas transplantation (PTX) in type 1 diabetic patients can sustain insulin independence for indefinite periods. Despite of insulin independence, not all successfully transplantated pancreas grafts are able to normalize glucose metabolism completely. Especially oral glucose tolerance test is often impaired or diabetic after pancreas transplantation. This is of relevance, since an impaired or diabetic glucose tolerance is associated with a poorer longterm graft survival. The aim of the study was to measure the incidence of an impaired or diabetic glucose tolerance in graft recipients after successful transplantation. In addition other aspects of glucose metabolism for example insulin secretion (glucose and arginine induced) and insulin resistance were analysed.
Materials and Methods: 167 Type 1 diabetic recipients (99 male, 68 female) of simultaneous pancreas kidney (SPK) transplants were investigated early after transplantation. Fasting glucose, HbA1c and renal function were assessed. In addition an oral glucose tolerance test was combined with an iv arginine test. Fasting insulin, BMI and the HOMA-IR served as parameters for insulin resistance.
Results: Despite of normal fasting blood glucose and HbA1c values only 64% of patients displayed a normal glucose tolerance (NGT), whereas 26% showed an impaired (IGT) and 10% showed an diabetic glucose tolerance (DGT). Fasting blood glucose and HbA1c values were significantly lower in patients with NGT compared to probands with IGT or DGT (FPG: 78 ± 11 mg/dl vs. 85 ± 11 mg/dl vs. 96 ± 13 mg/dl; $p<0.001$; HbA1c: 4.7 ± 0.1% vs. 4.9 ± 0.1% vs 5.4 ± 0.2%; $p<0.01$). Insulin secretion (area under the curve 0–120 min after glucose load) was significantly reduced in patients with IGT and DGT. Stimulation of insulin secretion induced by arginine was significantly higher in patients with NGT, however differences did not achieve statistical significance. Indicators of insulin resistance (BMI, fasting insulin, HOMA-IR) were not different between different grades of glucose tolerance.
Conclusion: Graft recipients with impaired or diabetic glucose tolerance displayed higher fasting blood glucose values and HbA1c levels. Abnormal glucose tolerance was associated with a reduced secretory response of the pancreas graft but not with insulin resistance.

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Immunogenetic characterisation of the LEW.1AR1-*iddm* rat

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Background and Aims: The LEW.1AR1-*iddm* rat is an animal model of human Type 1 diabetes mellitus (T1DM) which arose through a spontaneous mutation within the inbred strain LEW.1AR1. Earlier genetic analyses using a (BN x LEW.1AR1-*iddm*) x LEW.1AR1-*iddm* backcross population revealed three diabetes susceptibility loci (*Iddm1*, *8*, *9*). Recently, we ovbserved that the T-cell content in peripheral blood lymphocytes (PBL) within the LEW.1AR1-*iddm* population is altered compared to that of the coisogenic background strain. The mode of inheritance of this deviant phenotype is autosomal recessive. It was the aim of the study (a) to characterise the T-cell repertoire of the LEW.1AR1-*iddm* rat, (b) to identify the gene responsible for this deviant phenotype and (c) as well as the mutation causing T1DM.
Materials and Methods: T-cell content and ratio of CD4+/CD8+ T-cells in PBL were analysed using FACS™ analyses with monoclonal antibodies. 91 microsatellite markers were used for linkage analyses of 100 (BN x LEW.1AR1-*iddm*) x LEW.1AR1-*iddm* backcross animals.
Results: The T-cell content in PBL within the LEW.1AR1-*iddm* population varied within a range of 55 ± 1.45% (M±SEM) while the background strain showed a T-cell content of 69 ± 1.04%. The CD4+/CD8+ T-cell ratio in PBLs of diabetes-resistant LEW.1AR1 rats was on average 2.1 ± 0.02 with a minimum of 1.9 and a maximum of 2.3. The diabetic LEW.1AR1-*iddm* group had a significantly lower CD4+/CD8+ T-cell ratio in PBLs (1.52 ± 0.06), but the variability was greater (minimum 0.9, maximum 2.0) compared with the coisogenic LEW.1AR1 strain. Linkage analyses showed that the locus responsible for alteration of the T-cell content within the LEW.1AR1-*iddm* population was located at the distal end of RNO1 closely linked to *Iddm8* (LOD >11) flanked by *D1Rat295* and *D1Rat496* (213 Mb - 243 Mb). Approximately 133 genes were mapped in this region. Potential candidate genes are *Hmgb1* (228 Mb), which has cytokine function and the T-cell receptors *Cd5* (213 Mb) and *Cd6* (213 Mb).
Conclusion: The findings of the linkage analyses indicate that the mutation of the LEW.1AR1-*iddm* rat mapped within the *Iddm8* region at the telomeric end of RNO1. Moreover, this mutation is obviously responsible for alterations in the T-cell content of PBLs which may also lead to diabetes development. Interestingly, the main part of *Iddm8* on *RNO1q51-55* is homologues to HSA10q25 containing *IDDM17*, while a minor part at the centromeric end of *Iddm8* (*RNO1q42-43, D1Rat295 - D1Rat496)* is homologue to *HSA11q13* containing *IDDM4*. Thus, in conclusion, the LEW.1AR1-*iddm* rat is a valuable T1DM model to elucidate genetic and immunological factors responsible for disease manifestation.

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Effects and mechanisms of glumatic acid decarboxylase (GAD)65 DNA vaccine preventing diabetes in non-obese diabetic (NOD) mice

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Background and Aims: Type 1 diabetes results from autoimmune destruction of the insulin-producing beta-cells in the islets of Langerhans. It has been shown that the destruction of pancreatic β cells is mediated by both CD4+ and CD8+ T cells. GAD65 is one of key beta-cell autoantigens in NOD mice and human type 1 diabetes, and used to regulate these autoreactive T cells and prevent autoimmune type 1 diabetes in NOD mice. The aim of this study was to investigate the effects and mechanisms of human GAD65 DNA vaccine preventing insulitis and diabetes in NOD mice.
Materials and Methods: Female NOD mice at 4 weeks of age were randomly divided into PBS (n=21), pcDNA (n=20), and hGAD65 (n=21) groups. Mice in each group received two intramusclar injections of 0.05 ml PBS alone, 50 μg pcDNA3.1 and 50 μg DNA vaccine emulsified in 0.05 ml PBS 7 days apart respectively. The accumulative diabetes incidence was detected up to 30 weeks of age in each group of NOD mice. Pancreas was removed from NOD mice at 12 weeks of age in each group (n=10) to score insulitis severity by routine H-E staining. The apoptotic β cells in islets were observed with double-labeling technique of TUNEL in situ combined with standard sensitive avdin-biotin complex (sABC) immunohistochemical method. Their spleens were for cell culture and total RNA extraction. Spleen IL-4, IFN-γ, NF-ATc and NF-ATp mRNA levels were tested by RT-PCR. IL-4 and IFN-γ levels in sera and supernatants of spleen cells were measured by ELISA.
Results: (1) At 30 weeks of age, the diabetes incidence was 95.2%(20/21), 80.0%(16/20) and 61.9%(13/21) in PBS, pcDNA and hGAD65 group respectively. The diabetes incidence in the PBS group was higher than that in hGAD65 group (P=0.008). (2) At 12 weeks of age, the insulitis scores in hGAD65 group was lower than that in PBS group (p=0.001) and pcDNA group (p=0.027) respectively. (3) The apoptotic β cell rates in hGAD65 group were lower than that in PBS group (P=0.014) and pcDNA group (P=0.023). (4) IL-4 levels in sera, spleen IL-4 and NF-ATc mRNA level in hGAD65 group were higher than those in PBS group (p<0.05) and pcDNA group (p<0.05) respectively. NF-ATp mRNA level in hGAD65 group was lower than that in PBS group (p<0.05).
Conclusion: Human GAD65 DNA vaccine via down-regulating NF-ATp and upregulating NF-ATc and IL-4, makes Th cells deviate to Th2, and subsequently prevents insulitis, beta-cell apoptosis and diabetes onset in NOD mice.
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Oral probiotic administration in the NOD mouse induces systemic and islet IL-10 Production and down regulates pancreatic expression of proinflammatory cytokine and chemokines

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Background and Aims: Recent data show that the modulation of GALT (gut-associated lymphoid tissue) may represent a means to affect the natural history of autoimmune diabetes as they drew attention to a possible involvement of GALT in the pathogenesis of disease, mostly as a potential site of priming of diabetogenic cells. These mucosal integrin alpha4 beta7 positive cells are recruited into the islets by IFN->= induced, proinflammatory chemokines CXCL9 and CXCL10, that bind CXCR3 receptor on lymphocytes membrane. Oral administration of probiotic bacteria is able to immunomodulate the function of GALT and to influence systemic immune responses. In a previous study we demonstrated that oral administration of probiotic compound VSL#3 induced an immunomodulation characterized by increased IL-10 production by splenocytes and Peyer's Patches associated to diabetes prevention in NOD mice.
We aimed to investigate the effects of GALT immunomodulation, induced by oral administration of the probiotic compound VSL#3 (containing viable, lyophilized bifidobacteria, lactobacilli and Streptococcus salivarius), on the islet inflammatyory process in female NOD mice.
Materials and Methods: VSL#3 (3 mg resuspended in PBS) was administered by gavage to female NOD mice 3 times per week, from the 4th week of age. Control group received PBS. Pancreas were removed from 10 NOD mice per group at week 12. IL-10, IFN-gamma, TNF-alpha, CXCL9, CXCL10 and CXCR3 expression was evaluated by Real Time quantitative PCR in pancreas. Histological and immunohistochemical studies were performed to characterise insulitis.
Results: VSL#3 treated mice showed a reduced insulitis score (p=0.002) and a decreased rate of beta-cell destruction (p=0.019) evaluated by islet morphometric analysis of insulin-positive cells. In addition, VSL#3 treated mice pancreas were characterized by an increased expression of IL-10 (p=0.0005 vs PBS-treated mice), associated to a down-regulation of proinflammatrory cytokines IFN-gamma (p=0.024) and TNF-alpha (p=0.0485), proinflammatory chemokines CXCL-10 (p=0.0005) and CXCL-9 (p=0.0005) and of proinflammatory chemokine receptor CXCR3 (p=0.0005).
Conclusion: Oral VSL#3 administration to female NOD mice prevents diabetes onset and promotes IL-10 production not only at systemic level but also in the target organ, associated to a down-regulation of pancreatic proinflammatory cytokines and chemokines together with a reduction of recruitment of CXCR3-positive lymphocytes.

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Prozac protects mice from multiple-low-dose-streptozotocin induced diabetes

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Background and Aims: Multiple-low-dose-streptozotocin (MLDS) treatment of mice induces insulitis and progressive hyperglycemia. The antidepressant Prozac has been shown to be anti-inflammatory in a rat model of paw inflammation and mouse model of lung inflammation. Prozac also reduces inflammatory cytokine and chemokine production in mice treated with lipopolysaccharide. Therefore, the aim of this study was to determine if Prozac could protect against MLDS-induced diabetes.
Materials and Methods: Male BALB/c mice were treated with Prozac (10 mg/kg/day) orally, treatment starting on the same day as streptozotocin (stz). Diabetes was induced by IP injection of stz (40 mg/kg) on five consecutive days, blood glucose was monitored over a 21-day period. Pancreas samples were taken on day 21 to measure insulin content and cytokine/chemokine levels.
Results: The stz treated mice developed hyperglycemia progressivly over the 21 days, blood glucose levels of 92 ± 2 and 206 ± 15 mg/dl ($p<0.05$, n=24) on day 1 and 21 respectively, with 71% of the mice diabetic (blood glucose>200 mg/dl) on day 21. Prozac treatment protected against the stz-mediated increase in blood glucose and diabetes incidence, blood glucose level of 109 ± 10 mg/dl ($p<0.001$ vs. stz.), and diabetes incidence of 7% ($p<0.05$ χ^2 test) on day 21. Stz treatment decreased pancreas insulin content from 76 ± 7 to 12 ± 2 ng insulin/mg protein ($p<0.05$), whereas the Prozac treated mice had a significantly higher pancreatic insulin content 38 ± 4 ng insulin/mg protein ($p<0.01$ vs. stz). Stz treatment also significantly increased the pancreatic levels of the Th1 cytokines, IL-1 (from 1.9 ± 0.2 to 3.9 ± 0.6 pg/mg protein, $p<0.05$), IL-12 (from 3.9 ± 0.9 to 7.8 ± 1.2 pg/mg protein, $p<0.05$), and TNF-α (from 4.2 ± 0.6 to 7.6 ± 1.4 pg/mg protein, $p<0.05$) and the chemokine MIP-1α (from 0.6 ± 0.1 to 1.5 ± 0.2 pg/mg protein, $P<0.05$). Prozac treatment prevented the MLDS-mediated increases in pancreas levels of the cytokines and chemokine; IL-1 (1.9 ± 0.1 pg/mg protein, $p<0.05$ vs. stz), IL-12 (4.3 ± 0.2 pg/mg protein, $p<0.05$ vs. stz), TNF-α (3.8 ± 0.6 pg/mg protein, $p<0.05$ vs. stz) and MIP-1α (0.9 ± 0.1 pg/mg protein, $P<0.05$ vs. stz).
Conclusion: The anti-inflammatory effect of Prozac protects against development of type I diabetes in mice.

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High vs low islet cell antibody titer at onset of type 1 diabetes: differences in CD4+ T cells subsets and clinical course of the disease

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Background and Aims: The predictive value of islet cell antibody (ICA) levels at onset of type 1 diabetes regarding the changes in T cell subsets and clinical course of the disease has not yet been clarified. Therefore, the aim of this study was to compare (a) the percentage of memory (CD45R0+) and naive (CD45RA+) CD4+ T cell subsets at the onset of the disease and in the state of clinical remission (CR) and (b) duration of CR between two groups of patients with recent-onset Type 1 diabetes showing high ICA titer (80 Juvenile Diabetes Foundation (JDF) units) (group A, N=32) or low ICA titer (40 JDF U) (group B, N=30) at onset of the disease.
Materials and Methods: ICA levels were determined by indirect immunofluorescence on human pancreatic tissue sections and expressed in Juvenile Diabetes Foundation (JDF) units. The percentage of memory cell (CD45R0+) and naive (CD45RA+) CD4+ T cell subsets was analysed by using two-color immunofluorescence staining and flowcytometry. CR was defined as optimal metabolic control without insulin lasting >30 days.
Results: We found that there was no difference between the groups concerning the percentage of CD45R0+ or CD45RA+ subsets of CD4+ T lymphocytes (CD45R0+: 33.9+/-2.1 vs 34.6+/-2.0%, CD45RA+: 24.4+/-2.2 vs 24.8+/-2.1%, p=NS) at onset of disease. The percentage of CD4+CD45R0+ T cells significantly decreased in group B and it became significantly lower compared to group A in the state of CR (A:32.9+/-2.1 vs B:25.1+/-2.1%, $p<0.05$). Moreover, the percentage of CD4+CD45RA+ T cells increased in group B and it became significantly higher than to group A in the state of CR (A:21.4+/-1.8 vs B:26.6 +/-1.5, $p<0.05$). Simultaneously, in group A, the duration of CR was significantly shorter in group A than in group B (A: 259 ± 25 vs B: 447 ± 28days; $p<0.05$).
Conclusion: Our results have demonstrated that patients with recent-onset Type 1 diabetes and low ICA titer at onset of the disease have shown longer duration of CR together with the increase in naive (CD45RA+) and the decrease in memory (CD45R0+) CD4+ T lymphocyte subsets, in contrast to the patients with high ICA titer showing shorter CR without significant changes in the respective CD4+ T cell subsets. The results imply that the ICA levels at onset of the disease might have an important predictive value, being potential early markers of differences in the clinical course as well as in the changes of regulatory T cell subsets in recent-onset type 1 diabetes.

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Autoantibodies to antigens shared with the kidney and the pancreas type 1 diabetic patients

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Background and aims: Patients with type 1 diabetes typically develop autoantibodies to antigens of the pancreatic islet cells including insulin, glutamic acid decarboxylase and the IA-2 protein. Nephrin is a protein expressed both in the kidney glomeruli and pancreatic beta cells, and it has been shown to be affected in diabetic nephropathy. The appearance of circulating antibodies to nephrin has been detected in type 1 diabetic patients (unpublished data). Densin is a molecule expressed in the kidney glomeruli and according to our recent data in pancreatic beta cells as well. Therefore, we studied whether autoantibodies to densin appear in serum of diabetic patients. Our hypothesis is that pathogenesis of diabetes or diabetic nephropathy is associated with densin autoantibodies.
Materials and methods: We analysed 68 children and adolescents with type 1 diabetes and 91 healthy control subjects for densin autoantibodies using radioimmunoprecipitation assay. The serum samples were from a follow-up material gathered at the time of diagnosis of clinical diabetes and after disease duration of 2, 5 and 10 years.
Results: When all time points were included, densin autoantibodies were detected in 25% (17/68) of the type 1 diabetic patients, whereas only 3% (3/91) of the healthy control subjects showed positivity (p=0.001).
At the time of diagnosis, 19% (13/68) of the patients tested positive for densin autoantibodies, whereas 5% (3/62), 12% (7/59) and 2% (1/45) had these antibodies at 2, 5 and 10 years, respectively. This decrease during the course of the follow-up period was significant (p=0.001). Of the densin antibody positive patients 76% were ICA positive, 82% GADA positive and 82% IA2 positive whereas the percentages were 82%, 58% and 80% in densin antibody negative patients.
33% of the diabetic women and 20% of diabetic men in this study were densin autoantibody positive. All three healthy control subjects positive for densin autoantibodies were women.
Conclusions: In a subset of patients with type 1 diabetes circulating autoantibodies to densin are detected. The presence of these antibodies especially at the time of diagnosis could imply their role in the development of diabetes. Further studies to evaluate the association of densin autoantibodies with diabetic nephropathy are required.
Support: Finnish Diabetes Association

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Serum chemokine levels of CCL4/ MIP1β and CCL5/ RANTES are related to C-peptide and proinsulin levels in patients with newly diagnosed type 1 diabetes mellitus. Results from the Hvidøre Study Group

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Background and Aims: Diabetes mellitus type 1 results from immune-mediated destruction of β-cells and is accompanied by altered innate immune responses. Expression levels of the chemokine receptor CCR5 have been suggested as predictive biomarker in newly onset type 1 diabetes. The aim of the current study was to investigate circulating levels of the CCR5 ligands MIP-1β and RANTES and their relation with and prediction of residual β-cell capacity during the first 12 months after onset of type 1 diabetes in children and adolescents.
Materials and Methods: Serum was obtained from 256 newly diagnosed patients with type 1 diabetes (122 males/134 females, aged 9.6 years, range 0.2–16.8 years) recruited from 18 paediatric centers of the Hvidøre study group. HbA1c, stimulated C-peptide and proinsulin (Boost test) were deter-

mined by high pressure liquid chromatography (HbA1c) AutoDELFIA – fluoroimmunoassay (C-peptide) and sandwich ELISA (proinsulin). Serum cytokines and chemokines were measured by double sandwich ELISA. Data were analysed by multiple logistic regression analysis including gender, age, IL6, CCL4/MIP1β, CCL5/ RANTES and MIF as co-variates, non-parametric Wilcoxon test and Spearmans correlation analysis. All statistical analyses were performed using SAS version 6.12.

Results: One month after diabetes onset, HbA1c associated positively with CCL5/RANTES (coefficient: 0.009%/ng/ml, SE 0.002, p<0.0001), while C-peptide levels related negatively with CCL4/MIP1β (coefficient: –0.12%/pg/ml, SE 0.06, p= 0.0367). Six and twelve months after diabetes onset HbA1c was not related to any of the co-variates investigated. Six months after onset, C-peptide and proinsulin levels associated negatively with CCL4/MIP1β (coefficient: –0.13%/pg/ml, SE 0.07, p= 0.0588, coefficient: –0.28%/pg/ml, SE 0.09, p=0.018), while 12 months after onset, only proinsulin levels associated negatively with CCL4/MIP1β (coefficient: –0.31%/pg/ml, SE 0.09, p= 0.0020).

Prediction models for residual β-cell function (C-peptide and proinsulin) showed a negative association between CCL4/MIP1β levels at one month and C-peptide (coefficient –0.11%/pg/ml, SE 0.07, p= 0.08) and proinsulin (coefficient –0.28 SE 0.08, p= 0.0004) at 6 months and a negative association between proinsulin and CCL4/MIP1β levels (coefficient –0.37%/pg/ml, SE 0.01, p=0.0006) at 12 months. Furthermore CCL5/RANTES at one month associated positively with proinsulin (coefficient 0.53%/ng/ml, SE 0.25, p=0.03) at six months and proinsulin (coefficient: 0.76%/ng/ml, SE 0.29, p=0,0085) at 12 months.

Conclusion: The present study suggests that C-peptide and proinsulin levels are associated with innate immunity chemokines CCL4/MIP1β and CCL5/RANTES in the first year after diabetes manifestation. Furthermore the study indicates that CCL4/MIP1β and CCL5/RANTES may serve as predictive markers of disease progression and residual β-cell function during intervention trials in type 1 diabetes.

Support: Deutsche Diabetes Stiftung

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The effect of glycemic control on lymphocyte neural cell adhesion molecule (CD 56) expression in patients with type 2 diabetes mellitus

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Background and Aims: The CD 56 antigen is a 140 kDa isoform of the Neural Cell Adhesion Molecule (N-CAM). It is moderately expressed on a subpopulation of large granular peripheral blood lymphocytes and on cells with natural killer activity. Natural killer cells participate in the innate resistance to intracellular pathogens and malignancies and have a modulatory effect on adaptive immunity as well as hematopoiesis. We aimed to investigate relationship between glycemic control and natural killer activity in patients with type 2 diabetes.

Materials and Methods: Study population included 60 patients with type 2 diabetes. Patients with normal level of erythrocyte sedimentation rate and white blood cells were included in the study. Patients with any systemic disease and infection or patients using drugs known to affect on lymphocytes count were excluded from study. CD 56 expression was analyzed with flow cytometer. Glycemic control were evaluated with A1C levels. Patients were divided into groups as regard to the A1C levels (Group 1: A1C <7, Group 2: A1C ≥7 and < 10, Group 3: A1C ≥10).

Results: There was negative significant correlation between CD 56 expression and A1C level. CD 56 expression were higher in the group 1 when it's compared to group 2 and group 3 (Table 1).

Conclusion: The present study suggest that poor glycemic control is related with decreased natural killer activity in diabetic patients which may be part of impaired defense mechanism in these patients

Relationship between the glycemic control and cd56 expression in patients with type 2 DM

	Group 1 N=12	Group 2 N=28	Group 3 N=20	
Age (yr)	41,9 ± 7,4	49,9 ± 7,5	52,9 ± 9,6	p>0.05
BMI (kg/m^2)	28,2 ± 2,6	29,5 ± 2,8	29,5 ± 5,6	p>0.05
Fasting plasma glucose (mg/dl)	119,1 ± 11,2	182,2 ± 51,3	274,5 ± 91,2	p<0.001
Postprandial plasma glucose (mg/dl)	178 ± 58	280,5 ± 75,5	365,0 ± 126,6	p<0.001
A1C(%)	6,6 ± 0,79	8,1 ± 0,85	12,5 ± 1,75	p<0.001
CD56 (%)	18,4 ± 8,5**	13,2 ± 5,5*	11,7 ± 7,4	p=0.043* p=0.010**

Supported by Inonu University

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Methodological approaches to diabetes research

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Differentially displayed serum proteins of individuals with type 2 diabetes mellitus

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Background and Aims: Type 2 diabetes mellitus (T2DM) is a disease where the manifestations of the disease in the blood are not restricted to changes in levels of insulin and glucose. On the contrary, many specific blood borne proteins affecting the insulin secretory capacity have been identified, which implies the complexity by which blood proteins are altered during the progression of the disease. In the present study we have generated serum protein profiles of 10 individuals with normal glucose tolerance (NGT) and with high insulin secretory capacity (HOMA-beta) and of 10 individuals with T2DM and low HOMA-beta. The serum protein profiles were compared and analysed with the aim of identifying complex changes in protein expression patterns implicated in the development of the insulin secretory failure in T2DM.
Materials and Methods: The study subjects were selected from the Stockholm Diabetes Prevention Program (SDPP). The individuals were males without family history of T2DM. The NGT and T2DM groups were age- and weight-matched. Fasting serum samples were denaturated, diluted and applied on immobilized metal affinity capture (IMAC) protein arrays at pH 4. After washing and application of matrix, the arrays were read in a surface enhanced laser desorption/ionization time-of-flight mass spectrometer (SELDI-TOF MS). Mass spectra were analysed and differentially displayed peaks discovered.
Results: Mass spectra of serum obtained from individuals with NGT and T2DM were compared. A high number of peaks representing serum proteins were found on the IMAC arrays. Six of these proteins were differently expressed in the two groups (Mann-Whitney U test, $p<0.05$). These peaks represented proteins ranging from 16 to 74 kDa. Whereas three of these proteins were up-regulated, the remaining proteins were down-regulated in serum from T2DM individuals compared to NGT individuals. To identify these differentially displayed proteins, the complexity of serum samples was reduced by anionic fractionation. The eluates will now be subjected to one-dimensional gel electrophoresis, in gel trypsin digestion and peptide mass fingerprinting, which is expected to yield the identities of the proteins.
Conclusions: Insulin secretory failure leading to T2DM is a complex process, which involves expression alterations of many proteins. Some of these changes are evident in the blood; by protein profiling of serum samples from individuals with or without T2DM differentially displayed proteins were discovered. The identities of these proteins are in the process to be determined. The proteomic approach may give information relevant for the understanding of the molecular mechanisms of insulin secretory failure in T2DM.

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Identifying differentially expressed secreted novel peptides in diabetic skeletal muscle using a signal sequence trap

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Background and Aims: Secreted and membrane bound proteins play an important role in intercellular communication and are targets for the development of therapeutic agents. To identify novel and uncharacterised secreted or cell surface proteins involved in type 2 diabetes and insulin resistance a signal sequence trap (SST) was developed. The SST and cDNA microarrays was used to identify secreted and membrane bound proteins that are differentially expressed in red gastrocnemius muscle of lean, normal glucose tolerant (nGT), and obese, diabetic (T2D) *Psammomys obesus*.
Materials and Methods: A cDNA library enriched for 5' regions of *P. obesus* red gastrocnemius cDNAs was synthesised from red gastrocnemius muscle mRNA. These cDNAs were ligated into a retrovirus plasmid vector pLNCX2 5' upstream to a murine interleukin 3 (mIL-3) which was engineered to lack a signal sequence. This muscle plasmid library, representing 200,000 clones was transfected into a retrovirus packaging cell line and the retrovirus library produced was used to infect the mIL-3 dependent cell line FDCP1. Genomic DNA was extracted from clones growing in the absence of mIL-3 and cDNAs were isolated by nested PCR amplification. The PCR products were screened for differential expression by cDNA microarray analysis using red gastrocnemius muscle from nGT and obese T2D *P.obesus*. Microarray data was analysed using GenePix Pro 5.1 and Acuity 4.0 software (Axon Instruments). Differentially expressed clones were sequenced and gene expression was then confirmed by Real Time PCR (RT-PCR) in red gastrocnemius muscle from nGT and obese T2D *P.obesus* fed *ad libitum* or fasted for 24 hours.
Results: 1600 positive clones were generated from the SST, and initial sequencing of 139 clones identified 79 genes encoding 36 known membrane bound proteins, 13 known secreted proteins and 30 proteins not previously known to be secreted or membrane bound. Six of these proteins were predicted by SecretomeP to be secreted and may be involved in non-classical protein secretion. cDNA microarray analysis of gene expression identified 96 differentially expressed clones. These clones were sequenced to identify a total of 62 genes including 15 novel proteins not previously characterised. These 62 genes encode 32 secreted or membrane bound proteins, and 30 genes not previously known to be secreted or membrane bound. Examples of differentially expressed genes include CTRP2, adiponectin, calsequestrin, and SPARC. RT-PCR analysis of CTRP2 gene expression in fed or fasted red gastrocnemius was found to be significantly increased by 215% between nGT and T2D fasted animals ($p=0.012$). Expression of CTRP2 was significantly correlated with body weight ($r=0.37$, $p=0.015$) and plasma insulin levels ($r=0.23$, $p=0.040$).
Conclusion: SST coupled with cDNA microarray technology was used to identify differentially expressed secreted and membrane bound proteins in red gastrocnemius muscle of nGT and obese T2D *P. obesus*. A number of known and previously uncharacterised secreted proteins have been identified. These proteins represent new candidate targets for therapeutic treatment of type 2 diabetes.

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The assessment of glucose appearance rate and hepatic glucose uptake by [18F]fluorodeoxyglucose: validation against 6,6-2H-glucose

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Background and Aims: Imaging with positron emission tomography and [18F]fluorodeoxyglucose (FDG) provides quantitative information on regional glucose metabolism. We evaluated the suitability of plasma FDG measurements to estimate glucose rate of appearance (Ra) and liver glucose uptake.
Materials and Methods: The study was conducted in anaesthetised pigs, during fasting and euglycaemic hyperinsulinaemia (1 mU/min/kg). Catheters were placed in the carotid artery, portal vein and hepatic vein. Flow-meters were used to measure hepatic artery and portal vein blood flow. Blood samples were simultaneously collected from the three vessels for 180 minutes, after co-injection of 6,6-2H-glucose and FDG. Ra was calculated as: metabolic clearance rate x steady-state glycaemia (FDG) or injected dose / area of tracer-to-tracee ratio (6,6-2H-glucose). Liver uptake of the two tracers was assessed as arterial-portal-venous balance during the first five min after injection; for comparison, values were expressed as percentage of injected dose (%ID).
Results: In the fasting state, glucose Ra, as estimated with 6,6-2H-glucose and FDG was 3.5 ± 0.6 and 3.1 ± 0.4 mg/kg/min (19.5 ± 3.2 vs 17.0 ± 2.4 µmol/kg/min, ns), respectively. Corresponding values during the clamp were 4.3 ± 0.8 and 4.2 ± 0.2 mg/kg/min (24.1 ± 4.7 vs 23.1 ± 1.0 µmol/kg/min, ns). Liver uptake rates of 6,6-2H-glucose and FDG were 1.2 ± 0.5 and 1.1 ± 0.3%ID/min (5.9 ± 2.6 and 5.4 ± 1.6%ID over the 5-min measurement period, ns), respectively.
Conclusion: We conclude that plasma FDG kinetics provides an accurate estimate of glucose Ra and, thus, can be used to quantify endogenous glucose production. In addition, FDG and glucose are similarly retained by the liver early after injection.

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Noninvasive measurement of the contribution of hepatic glycogenolysis to fasting glucose production in type 1 diabetics by ^2H_2O and paracetamol

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Background & Aims: Hepatic glycogen has a key role in daily glycemic control as a sink for excess carbohydrate during feeding and a source of endogenous glucose during fasting. In type 1 diabetics (T1D), rates of hepatic glycogen synthesis during feeding and glycogenolysis during fasting are often diminished. One key consequence is that fasting glucose production becomes more dependent on gluconeogenesis which may increase the likelihood of fasting hypoglycemia. Recent *in vivo* ^{13}C NMR studies have shown that for TID patients, hepatic glycogen synthesis during feeding and hydrolysis to glucose during fasting can both be restored to normal values by intensive insulin therapy. These studies demonstrate that hepatic glycogen metabolism measurements could provide useful information for the evaluation of glycemic control therapies in TID patients. We present a practical assay of hepatic glycogenolysis using Landau's ^2H_2O measurement of endogenous sources of glucose production that is based on the analysis of urinary Paracetamol glucuronide ^2H enrichment rather than that of plasma glucose. The fractional contribution of glycogenolysis to endogenous glucose production in T1D subjects and healthy controls was determined with this method.
Materials &Methods: Nine healthy subjects (5M, 4F, Hb_{1Ac} 4.9 ± 0.2) and seven TID patients (6M, 1F, Hb_{1Ac} 7.1 ± 1.6) of matching ages and BMI were studied. Subjects began fasting at 20:00. During the night, 1000 mg Paracetamol and 3.0 g/kg body-water ^2H_2O were given at 02:00. Urine was collected between 06:00–08:00 the next morning and urinary glucuronide was derivatized to monoacetone glucose. In this form, the enrichment of position 5 relative to position 2 (D5/D2) was quantified by ^2H NMR. The percent contribution of glycogenolysis to endogenous glucose production was estimated as 100 x [1-(D5/D2)]. Body water ^2H-enrichments were quantified by ^2H NMR analysis of urine water. Data are reported as mean values±standard deviation.
Results: Body water ^2H-enrichments were identical for controls and TID subjects (0.27 ± 0.03% and 0.27 ± 0.02%, respectively). The glucuronide D5/D2 of healthy subjects was 0.55 ± 0.05, corresponding to a 45 ± 5% contribution of hepatic glycogenolysis to endogenous glucose production. This consistent with recent NMR and MS measurements of plasma glucose enrichment from ^2H_2O in overnight fasted healthy subjects. In comparison, the TID group had significantly higher glucuronide D5/D2 values (0.63 ± 0.08, $p < 0.05$ vs. controls) corresponding to a significantly lower contribution of hepatic glycogenolysis to endogenous glucose production (37 ± 8%, $p < 0.05$ vs. controls). These values are consistent with *in vivo* ^{13}C NMR measurements of glycogenolysis in TID patients.
Conclusions: Hepatic glycogenolysis in TID patients can be noninvasively assayed in a standard hospital setting by analysis of urine following ingestion of ^2H_2O and Paracetamol. These measurements reveal a significant reduction in the contribution of glycogenolysis to fasting glucose production in a group of T1D patients compared to healthy controls. The ^2H_2O-Paracetamol measurement of glycogenolysis can be easily incorporated with existing clinical evaluations of TID subjects.
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Changes in hepatic glycogen cycling during a glucose load in healthy humans

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Background and Aims: Glycogen cycling (GC), i.e. simultaneous glycogen (GLY) synthesis and breakdown, affects estimates of glucose fluxes using tracer techniques and may contribute to hyperglycemia in diabetic conditions. This study presents a new method for quantifying hepatic GC in the fed state. GLY is synthesized from glucose by the direct (D) and indirect (gluconeogenic, I) pathways. To GC's extent, since GLY is also synthesized from GLY, i.e. GLY→G1P→GLY, less than 100% of GLY is synthesized by I+D, i.e. %GC=100-(I+D).
Materials and Methods: I and D were measured independently in nine overnight fasted healthy volunteers. They ingested ^2H_2O (5 ml/kg body water) and were infused with [5-^3H]glucose and acetaminophen (1 g) during hyperglycemic clamps (7.8 mM) lasting 8 h. The percent contribution of I was calculated from the ratio of ^2H enrichments at carbons 5 to 2 and of D from the ^3H specific activity, relative to plasma glucose, of the urinary glucuronide excreted between 2-4, 4–6, and 6–8 h.
Results: Glucose infusion rates increased ($p<0.01$) to ~50 $\mu mol \cdot kg^{-1} \cdot min^{-1}$. Plasma insulin and the insulin:glucagon ratio rose ~3.6- and ~8.3-fold ($p<0.001$), respectively. From the difference between 100% and the sum of D (2-4 h: 54 ± 6%; 4–6 h: 59 ± 5%; 6–8 h: 63 ± 4%) and I (32 ± 3; 38 ± 4; 36 ± 3%) pathways, GC decreased ($p<0.05$) from 14 ± 4% (2–4 h) to 4 ± 3% (4–6 h) and 1 ± 3% (6–8 h).
Conclusion: This method allows measurement of hepatic GC in the fed state and demonstrates that GC occurs most in the early hours upon glucose loading following a fast.

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Use of a stable isotope technique to estimate postprandial chylomicron triacylglycerol concentrations without physical separation of the particles

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Background and Aims: Increased postprandial concentrations of gut-derived chylomicron- and liver-derived VLDL-triacylglycerol (TG) are associated with insulin resistance and type 2 diabetes, yet there are no simple methods currently available for their measurement. Density gradient ultracentrifugation may be used to separate particles in the Svedberg flotation rate $S_f > 400$ (chylomicron-rich) and S_f 60-400 (VLDL-rich) lipoprotein fractions. However, there is a considerable proportion of small chylomicron particles and chylomicron remnants in the S_f 60-400 fraction. The aim of this investigation was to estimate chylomicron-TG concentrations from plasma concentrations of [U-^{13}C]palmitic acid given orally. The results were then compared with TG concentrations measured in the $S_f > 400$ fraction.
Materials and Methods: Healthy subjects (n=7, fasting TG 1.0–3.3 mmol/L) were studied. Subjects ingested 100 mg [U-^{13}C]palmitic acid in a mixed meal of known fatty acid composition. Blood samples were taken prior to isotope ingestion and for the next 6 h. Isotopic enrichments in plasma TG were measured by gas chromatography-mass spectrometry (GC-MS), and plasma fatty acid compositions were measured by GC. TG concentrations were measured in total plasma and in the $S_f > 400$ fraction by enzymatic assay.
Results: Chylomicron-TG concentrations were calculated from plasma concentrations of [U-^{13}C]palmitate-TG. The calculation was based on the following assumptions: a) The enrichment of [U-^{13}C]palmitate-TG and fatty acid composition of chylomicrons were both equal to the test meal. b) Most [U-^{13}C]palmitate-TG was associated with gut-derived chylomicron (apoB-48-containing) particles. The second assumption is limited by the fact that in the postprandial period, VLDL derived from dietary chylomicron remnants and non-esterified fatty acids will also contain [U-^{13}C]palmitate-TG. However, we have previously found that up to 20% of meal-derived fatty acids were incorporated into VLDL 5 h after a meal. Chylomicron-TG concentrations measured by density gradient ultracentrifugation ($S_f > 400$ fraction) were significantly (39–52%, $P<0.001$) lower than the estimated value from stable isotope measurement in the period up to 5 h following the meal.
Conclusion: Despite inherent assumptions, the use of a simple stable isotope technique using plasma sampling is potentially a more accurate estimate of chylomicron-TG concentrations than density gradient ultracentrifugation, in the early postprandial period. This model would also allow for estimation of liver-derived VLDL-TG concentrations.
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Assessment of insulin sensitivity and beta-cell responsivity by oral minimal models: mixed meal against OGTT

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Background and Aims: The Oral Glucose Tolerance Test (OGTT) and the Meal Glucose Tolerance Test (MGTT) became recently widely used tests to assess insulin sensitivity (S_I), β-cell responsivity to glucose level (static responsivity, Φ_s) and its rate of change (dynamic responsivity, Φ_d) and thus disposition indices (static: $DI_s=S_I \cdot \Phi_s$; dynamic $DI_d=S_I \cdot \Phi_d$) in physiological conditions. Both OGTT and MGTT employ the oral route of glucose administration but, while the OGTT contains only glucose, the MGTT contains other nutrients, such as proteins and fat, which slow down glucose absorption rate and could affect the estimate of metabolic indices. The ability of the two tests in assessing insulin action and secretion in response to an oral glucose stimulus was so far never evaluated. The aim of this study is thus to compare estimates of metabolic indices obtained with OGTT and MGTT in the same subjects in order to assess whether or not possible discrepancies between the two methodologies occur.
Materials and Methods: Eight subjects with normal fasting glucose (G_b= 90.23 ± 1.64 mg/dl) and ten with impaired fasting glucose (G_b= 113.01 ± 2.42 mg/dl, p<0.0001) (age= 53 ± 2 years; BMI =30.03 ± 0.99 kg/m^2) received both an MGTT (10 kcal/kg, 45% carbohydrate, 15% protein, 40% fat, 75 g of glucose) and a 75 g of glucose OGTT; plasma samples were frequently collected in the 240 minutes following the oral ingestion and plasma glucose, insulin and C-peptide concentrations were measured thus allowing to estimate S_I and Φ_s, Φ_d, DI_s and DI_d from Oral Glucose and C-peptide Minimal Models.
Results: A good agreement between MGTT and OGTT metabolic indices was found both in terms of value (no difference was found by Wilcoxon Signed Rank Test, significance level set to 0.05) and correlation: S_I^{MGTT}=7.06 ± 1.42 vs S_I^{OGTT}=8.97 ± 1.62 10^{-4} dl/kg/min per μU/ml (R= 0.81, p<0.0001); Φ_s^{MGTT}=32.47 ± 2.70 vs Φ_s^{OGTT}=31.93 ± 2.89 10^{-9} min^{-1} (R=0.75, p=0.0003); Φ_d^{MGTT}=543.56 ± 69.11 vs Φ_d^{OGTT}=542.24 ± 92.48 10^{-9} (R=0.89, p<0.0001); DI_s^{MGTT}=353.31 ± 61.73 vs DI_s^{OGTT} = 445.58 ± 84.01 10^{-14} dl/kg/min^2 per pmol/l (R=0.77, p=0.0002); DI_d^{MGTT}=5978.88 ± 1233.32 vs DI_d^{OGTT}=7308.17 ± 1313.42 10^{-14} dl/kg/min per pmol/l (R=0.79, p<0.0001).
Conclusions: Our results show that OGTT and MGTT provide equivalent results when used to estimate insulin sensitivity and β-cell responsivity to glucose by Oral Minimal Models in a wide spectrum of glucose tolerance; the two tests can thus be used interchangeably to assess the efficiency of glucose-insulin regulatory system in physiological conditions.

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Lifestyle intervention in human

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Changes of adiponectin oligomer composition by moderate weight reduction

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Background and Aims: Adiponectin affects lipid metabolism and insulin sensitivity, which are characteristically impaired in individuals with metabolic syndrome or obesity. Adiponectin is decreased in obese persons, while high levels protect against type 2 diabetes mellitus. However, adiponectin circulates in three different oligomers, which may also have distinct biological functions. The role of these oligomers in obesity and lipid metabolism after weight reduction has not been determined in detail yet.
Materials and Methods: 17 obese volunteers (15 women and 2 men) participated in a weight reduction programme for 6 months. Hyperinsulinemic euglycemic clamps, detailed anthropometric examinations and laboratory analyses were performed before and after 6 months of a balanced diet (50% carbohydrates, 30% fat, 20% protein). Calorie intake was recommended to be 400–600 kCal below the individual basal metabolic rate. Adiponectin was determined by ELISA, oligomers were detected by non-reducing, non-heat denaturating Western blot and were associated with anthropometric and metabolic characteristics. Percentage adiponectin oligomers were multiplied with total adiponectin levels to calculate absolute oligomers values.
Results: BMI was reduced (35.1 ± 1.2 to 32.8 ± 1,1 kg/m^2; p<0.001), which was associated with an improved metabolite profile. HDL cholesterol increased significantly (1.28 ± 0.06 to 1.36 ± 0.06 mmol/l; p=0.029). Total cholesterol (5.7 ± 0.2 to 5.9 ± 0.2 mmol/l, p=0.305), LDL cholesterol (3.7 ± 0.1 to 3.8 ± 0.1 mmol/l, p=0.693), triglycerides (1.6 ± 0.1 to 1.6 ± 0.1 mmol/l, p=0.699), free fatty acids (0.74 ± 0.05 to 0.63 ± 0.06 mmol/l, p=0.121), HbA1c (5.4 ± 0.1 to 5.4 ± 0.1%, p=1.0) and M-Value (2.9 ± 0.3 to 3.2 ± 0.2, p=0.320) changed, but not significantly. Total adiponectin raised from 5.3 ± 0.5 to 6.1 ± 0.6 μg/ml (p=0.076). Interestingly High and Medium Molecular Weight adiponectin oligomers (HMW and MMW) increased during weight reduction (HMW: 6.7 ± 0.9 to 7.9 ± 0.7%, p = 0.042; MMW: 43.1 ± 1.4 to 46.1 ± 1.5%, p = 0.007), which was accompanied by inverse, although non-significant, changes of Low Molecular Weight oligomers (LMW: 50.0 ± 1.8 to 45.8 ± 1.7%). We found no significant correlation between total adiponectin and BMI (r=-0.258; p=0.354) but an inverse correlation between HMW and BMI (r=-0.695; p=0.002) and a positive correlation between LMW and BMI (r=0.579; p=0.015). Total adiponectin correlated positively with HDL cholesterol (r=0.61; p=0.016), which is also reflected by the positive correlation between HDL and absolute HMW (r=0.665; p=0.007), absolute MMW (r=0.604; p=0.017) and absolute LMW (r=0.536, p=0.039). Multivariate linear regression analysis demonstrated that absolute HMW and FFA values before weight reduction predicted about 60% of HDL cholesterol changes during weight loss.
Conclusion: We conclude that weight reduction results in a relative increase of HMW/MMW adiponectin and a reduction of LMW adiponectin. Total adiponectin and especially HMW adiponectin are related to circulating HDL cholesterol.
Support: German Diabetes Association

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Soluble CD14 circulates in proportion to adiposity in healthy subjects and decreases after weight loss

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Background and Aims: Soluble CD14 (sCD14), detectable at high concentrations constitutively present in the circulation, plays a key role in the neutralization of lipopolysaccharide (LPS). CD14 mRNA has been demonstrated in adipose tissue. We aimed to investigate circulating sCD14 in relation to adiposity measurements in two different situations: a) apparently healthy subjects b) morbid obese patients before and after weight loss.

Materials and Methods: Inclusion criteria for apparently healthy subjects were: 1) BMI <40 Kg/m^2, 2)absence of any systemic disease 3) absence of any infection in the previous monthMorbid obese patients were evaluated before and two years after bilio-pancreatic diversion. Circulating sCD14 was measured using ELISA. Body composition was evaluated by bioelectric impedance (healthy subjects) and tritiated water (morbidly obese subjects). Insulin sensitivity before and after weight loss was evaluated using euglycaemic hyperinsulinaemic clamp.
Results:
a) Healthy subjects: Age: 38.7 ± 9.8 years, 93 women (BMI:24.6 ± 4.3Kg/m^2, percent fat mass:24.9 ± 10.4, WHR:0.84 ± 0.05), 53 men (BMI: 25.7 ± 3.1Kg/m^2, percent fat mass:18.4 ± 10.5, WHR:0.96 ± 0.05). Women showed significantly higher circulating sCD14 concentrations than men (4.8 ± 1.8 vs 4.1 ± 1,8 µg/ml), in parallel to increased percent fat mass. sCD14 was significantly associated with both absolute and percent fat mass (r=0.19, p=0.02 and r=0.20, p=0.01, respectively) and these relationships were stronger in women (r=0.31 and r=0.32, respectively) than men.
b) Morbid obese patients: 11 patients (8 women, 3 men) were surgically operated for morbid obesity, BMI decreased from 43 ± 6.5 to 32.2 ± 5.8 (p<0.0001). The change in circulating sCD14 was significantly associated with the change in BMI (r=0.80, p=0.003).
Insulin sensitivity (M clamp value) increased after weight loss (from 2.35 ± 0.95 to 6.76 ± 0.8 mg/kg/min, p<0.0001). Interestingly, the change in sCD14 was also inversely associated with the increase in insulin sensitivity (r=-0.59, p=0.03).
Conclusion: Circulating sCD14, an important inflammatory mediator modulating LPS responses, is associated with adiposity in healthy subjects and decreases significantly after weight loss in parallel to improved insulin sensitivity.

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The changes of chronic inflammatory molecules and insulin signal transduction in type 2 diabetes mellitus after weight reduction
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Background and Aims: To study the change in insulin signaling in obese patients with type 2 DM after weight reduction
Materials and Methods: We assessed chronic inflammatory molecules (e. g., TNF-a, IL-6, IL-10, leptin, adiponectin, resistin) and the insulin-signaling cascade, including the insulin receptor(IR-β), insulin receptor substrate(IRS)-1, Akt, GSK-3, and PKC-ξ, -λ, -θ, -δ, -α, and -β phosphorylation before and after insulin stimulation by euglycemic hyperinsulinemic clamp test in 15 normal controls and 13 type 2 DM patients. Also eight of the type 2 DM patients repeated the exam after 6 months of diet and exercise.
Results: There were significant differences in the levels of chronic inflammatory molecules between the controls and DM groups, especially IL-6 and adiponectin. The euglycemic hyperinsulinemic clamp test showed a decreased glucose utilization rate in the DM group compared with the controls (p<0.05). GLUT 4 translocation from the cytosol to the plasma membrane on insulin stimulation was reduced in the DM group compared with the control group(p<0.05). Moreover, GLUT 4 translocation on insulin stimulation was reduced in the baseline DM group compared with the follow-up DM group (p<0.05). The incremental rate of IR-β, IRS, Akt, and GSK-3 phosphorylation on insulin stimulation was reduced in DM compared with the controls (p<0.05). The incremental rate of PKC-ξ, -λ, -δ, -θ and -α protein on insulin stimulation was reduced in the DM group. By contrast, the incremental rates of PKC- β phosphorylation on insulin stimulation were comparable in the two groups. After weight reduction, we observed significant changes in IL-10 and resistin. Moreover, there was a significant improvement in membranous GLUT 4 translocation and insulin receptor, IRS-1, Akt, and PKC-ξ/λ phosphorylation in the diet-exercise group.
Conclusion: We concluded that weight reduction owing to exercise improves insulin resistance by improving insulin receptor, IRS-1, Akt, and PKC-ξ/ λ phosphorylation.

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Weight reduction increases skeletal muscle and adipose tissue but not myocardial insulin sensitivity. Study with positron emission tomography
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Background and Aims: Very-low-calorie diet (VCLD) has been shown to decrease weight and improve whole body insulin sensitivity. In this study we evaluated the tissue specific effect of weight reduction on insulin stimulated glucose uptake (GU) using 18F-labelled fluoro-deoxyglucose (FDG) and positron emission tomography (PET).
Materials and Methods: 16 healthy obese (BMI 33 kg/m^2) subjects underwent VLCD for 6 weeks. Femoral skeletal muscle and subcutaneous adipose tissue and myocardial glucose uptake were measured during euglycemic clamp and FDG-PET before and a week after diet intervention.
Results: In comparison to the baseline, VLCD significantly decreased weight (from 96 kg to 85 kg, p<0.0001). Whole body insulin sensitivity increased by 33% (p<0.01). This was mostly explained by the increase in skeletal muscle GU (+48%, p<0.02). Higher improvement in insulin sensitivity was observed in subcutaneous adipose tissue GU (+69%, p<0.03). In contrast, myocardial GU remained unchanged. Increase in whole body insulin sensitivity was associated with increase in subcutaneous adipose tissue GU (r=0.71, p=0.002), and increase in skeletal muscle GU (r=0.90, p<0.0001).
Conclusion: Rapid weight reduction enhances whole body insulin sensitivity by enhancing adipose tissue and skeletal muscle glucose uptake whereas myocardial glucose uptake remains unchanged.

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High visceral adipose tissue mass at baseline predicts improvement of glucose tolerance and insulin sensitivity in a lifestyle intervention programme
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Background and Aims: Visceral adipose tissue (VAT) is associated with features of the metabolic syndrome and increased liver fat content. While this association is well documented in several cross-sectional studies, it is unclear whether VAT and liver fat are of predictive value for the individual response to a lifestyle intervention.
Materials and Methods: We prospectively studied a cohort of 35 males and 49 females selected from a cross-sectional study (51 males and 69 females, age 45 ± 12 years, BMI 29.5 ± 4.8 kg/m^2 (mean±SD). At baseline, all subjects underwent determination of body fat depots (VAT and non-visceral adipose tissue (NVAT)) using whole body magnetic resonance (MR) imaging. Liver fat content was measured by MR spectroscopy. Insulin sensitivity was estimated from oral glucose tolerance test (oGTT) and additionally measured at baseline by an euglycemic hyperinsulinemic clamp in the cross-sectional study.
Results: In the cross-sectional study both VAT (R= –0.56, P<0.0001) and liver fat (R= –0.6, P<0.0001) were negatively correlated to insulin sensitivity measured by clamp. With lifestyle intervention BMI was reduced (from 29.6 ± 0.5 to 28.8 ± 0.5 kg/m^2, P<0.001) and both insulin sensitivity (12.7 ± 0.8 vs. 14.8 ± 0.9, P<0.01) and plasma glucose area under the curve (16.3 ± 0.3 vs. 15.3 ± 0.3 mmol/l/h, P<0.01) were improved. Post-intervention insulin sensitivity and plasma glucose area under the curve were predicted by the basal amount of VAT (for both parameters P<0.03) after adjusting for sex, age, pre-intervention insulin sensitivity and adiposity at baseline and follow up.
Conclusion: Our data suggest that VAT and liver fat content are independently associated with important determinants of glucose metabolism. In addition, VAT predicts glucose levels during the oGTT and insulin sensitivity, both cross-sectionally and prospectively during lifestyle intervention. Therefore, VAT might be a usefull predictor for the success of a lifestyle intervention program.

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Natural course of glucose tolerance and outcome of lifestyle intervention: role of insulin secretion and insulin sensitivity

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Background and Aims: Longitudinal studies indicate that worsening glucose tolerance (GT) is associated with a reduction in insulin secretion (ISEC) relative to insulin sensitivity (ISEN) (so called disposition index (DI)). It is unclear whether changes in DI predict improving and worsening of GT during both non-intervention and following lifestyle intervention.
Materials and Methods:. We studied 60 non-diabetic subjects (24 males, 26 females, 50 NGT, 10 IGT, age 41 ± 10 years, BMI 30.3 ± 5.5 kg/m^2) at baseline (B), after a follow-up time of 3.0 ± 1.8 years without intervention (-LI) and, subsequently, after 9 months of lifestyle intervention (+LI) with diet and physical activity. At every time point ISEN and ISEC were estimated from OGTT using validated indices.
Results: During the initial follow up period without intervention, GT deteriorated (6.36 ± 0.2 vs. 6.89 ± 0.2 mM, p=0.01) with no significant changes in body weight. Lifestyle intervention improved GT (6.89 ± 0.2 vs. 6.33 ± 0.2 mM, p<0.001), and reduced body weight (30.7 ± 0.7 vs. 29.8 ± 0.7 kg/m2, p<0.001). In subjects with improving glucose tolerance during the whole follow up period (non-progressors, n=27), DI remained unchanged from B: 12480 ± 1165 to -LI: 13423 ± 1142 arbitrary Units (p=0.25) and subsequently increased to +LI: 17272 ± 1547 aU (p=0.03). No change was observed in subjects with worsening glucose tolerance (progressors, n=33) (B: 15107 ± 1125 to -LI: 14143 ± 1297 (p=0.13) and subsequently +LI: 15425 ± 1460 aU (p=0.27). The change in DI and the change in ISEC was significantly different between progressors and non-progressors (p<0.01 for time x group effect).
Conclusion: We conclude that lifestyle intervention reverses the deterioration of GT occurring during non-intervention. Subjects improving their GT are able to improve insulin secretion relative to insulin sensitivity especially after lifestyle intervention. Worsening of glucose tolerance is associated with failure to increase insulin secretion relative to sensitivity during both natural follow up and lifestyle intervention.

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Effect of high and low sucrose diets on insulin action in man: a randomised controlled trial

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Background and Aims: The long-term impact of dietary carbohydrate, in particular sucrose, on insulin action and the development of diabetes and atherosclerosis is not established. Current guidelines advise restriction of sucrose intake to 10% of total energy intake.
Materials and Methods: We investigated the effect of a high *versus* low sucrose diet (25% *versus* 10% of total energy intake). Thirteen healthy male volunteers were included in a randomised crossover design with sequential 6-week dietary interventions separated by 4-week wash-out period. Mean age (±sem) was 34 ± 3 years, BMI 26.7 ± 0.9 kg/m^2, waist circumference 91.9 ± 3.0 cm, blood pressure 127/70 ± 3/3 mmHg. Median fasting insulin (lower quartile, upper quartile) was 10.2 (5.4, 11.0) mU/L and HOMA-IR 2.1 (1.1, 2.4). Weight maintenance diets with constant macronutrient profiles and fibre content were designed using the dietary analysis program WISP. Subjects were monitored and all food was weighed and distributed 3–5 times weekly. Energy intake was the same in both dietary periods. Insulin action was assessed using a 2-step euglycaemic clamp with the isotope dilution method (3-^{3}H glucose) used to determine glucose utilisation and endogenous glucose production rates. Results are described as mean±sem. Variables which were not normally distributed are described as median (lower quartile, upper quartile) and were transformed by logarithm prior to analysis. Analysis was by the method of Hills and Armitage for 2-period crossover studies.
Results: There was no change in weight or physical activity during the study period. An adverse effect on the fasting lipid profile was noted. Total cholesterol (4.62 ± 0.92 mmol/L *vs*. 4.01 ± 0.80 mmol/L, p<0.01) and LDL cholesterol (2.78 ± 0.30 *vs*. 2.25 ± 0.25 mmol/L, p<0.01) were higher after 25% sucrose compared to 10% sucrose. HDL cholesterol (1.20 ± 0.06 *vs*. 1.21 ± 0.06 mmol/L) was similar on the two diets whereas serum triglyceride (0.95 (0.66, 1.58) *vs*. 0.92 (0.66, 1.17) mmol/L, p=0.08) tended to be higher after 25% sucrose. Fasting endogenous glucose production was similar on the 25% and 10% sucrose diets (11.0 ± 0.8 *vs*. 11.1 ± 1.0 µmol/kg/min). At an insulin infusion rate of 0.4 mU/kg/min, there were no differences noted in endogenous glucose production (7.1 ± 1.1 *vs*. 6.1 ± 1.0 µmol/kg/min) or in peripheral glucose utilisation (19.6 ± 1.8 *vs*. 19.4 ± 1.7 µmol/kg/min). At an insulin infusion rate of 2 mU/kg/min, endogenous glucose production rates were similar (7.5 ± 1.0 *vs*. 5.0 ± 1.6 µmol/kg/min, p=0.23) whereas the peripheral glucose utilisation after 25% sucrose diet was 57.0 ± 4.9 µmol/kg/min and was greater than that after the 10% sucrose diet at 47.6 ± 3.6 µmol/kg/min (95% confidence interval for the increase in insulin sensitivity was –1.6 to 19.0, p=0.09).
Conclusion: In this study, a high-sucrose intake as part of a eucaloric, weight-maintaining diet had no detrimental effect on insulin sensitivity in healthy non-diabetic subjects. A number of adverse changes in serum lipids occurred, including increased total cholesterol, low-density lipoprotein cholesterol and triglyceride levels. It is unclear whether these changes relate to differences in dietary sucrose or fat content.
Support: The Sugar Bureau

PS 37
Incretins

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GLP-1 and Exendin-4 increase post-prandial leptin in type 2 diabetes
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Background and Aims: Incretins GLP-1 and exendin-4, in addition to their glucose-dependent stimulation of insulin secretion, reduce appetite, increase satiety and reduce body weight. Exendin-4 has been shown to increase the potent anorexigen leptin in streptozotocin diabetic, but not non-diabetic rats, but a 6 hour GLP-1 infusion did not change leptin levels in healthy volunteers. However, the effect of these incretins on plasma leptin has not been reported in humans with diabetes. We aimed to determine whether i.v. GLP-1 or subcutaneous exendin-4 affect plasma leptin during a standard two meal day profile in subjects with type 2 diabetes.
Materials and Methods: Eight diet and seven sulphonylurea treated subjects with type 2 diabetes (BMI 28.8 ± 1.0 and 30.2 ± 1.3 kg/m^2, respectively) were admitted on four occasions and received, in randomised order, either (i) saline infusion & s/c injections (SAL), (ii) GLP-1 infusion (1.2 pmol/kg/min) and saline s/c injections (GLP), (iii) saline infusion and s/c exendin-4 (4 µg/m^2) at 18.30 and 07.00 (BD-Ex) or (iv) saline infusion and s/c exendin-4 (2 µg/m^2) at 18.30, 22.00, 07.00 and 12.00 (QDS-Ex). Plasma glucose and leptin were measured from 0500 to 1745. Aggregate concentrations of analytes were defined as the time-dependent means for the following periods: Basal (0500–0700) (Basal), Daytime postprandial (0700–1745) (DayPP), Breakfast postprandial (0700–1200) (BPP), Lunch postprandial (1200–1745) (LPP). Each treatment arm was compared with the SAL control day. Variables were log transformed when necessary for parametric analyses and expressed as either mean (±SEM) or geometric mean (1SEM range).
Results: Basal glucose concentrations were reduced by QDS-Ex and GLP-1 (all p<0.01); daytime postprandial glucose was significantly reduced by all treatments (all p<0.001) Plasma leptin on the SAL day was 615 (506–747), 538 (449–646), 623 ± 99 and 622 ± 100 pmol/l for basal, daytime, breakfast and lunch periods, respectively. No treatment was associated with a change in basal plasma leptin (all p>0.3). However, plasma leptin was increased significantly over the whole daytime period, after breakfast by each treatment (table 1) and by GLP-1 and QDS-Ex after lunch. There were no differences in DayPP leptin increments between the diet and the sulphonylurea group, 113 (109–118)% vs 117 (112–123)%.
Conclusion: These results, not previously described in human subjects with diabetes, are consistent with responses observed in diabetic rats. While the increase we observed in plasma leptin associated with GLP-1 and Exendin-4 was not large, it is possible that this might contribute to the weight loss associated with therapy with these incretin peptides. These data merit further investigation.

Table 1: Leptin concentrations (pmol/l)

	SAL	GLP-1	BD-Ex	QDS-Ex
Leptin basal	615 (506–747)	637 (532–764) *p=0.52*	649 (543–775) *p=0.34*	667 (564–787) *p=0.29*
Leptin dayPP	509 (426–607)	599 (505–709) *p=0.011*	577 (492–676) *p=0.022*	583 (496–684) *p=0.01*
Leptin BPP	623 (± 99)	710 (± 105) *p=0.006*	691 (± 100) *p=0.032*	688 (± 100) *p=0.031*
Leptin LPP	622 (± 100)	723 (± 109) *p=0.006*	669 (± 102) *p=0.19*	684 (± 94) *p=0.018*

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Effect of 4 weeks of near-normalization of blood glucose on beta-cell sensitivity to glucose and GLP-1 in type 2 diabetic patients
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Background and Aims: The beta-cell responsiveness to GLP-1 is impaired in patients with type 2 diabetes (T2DM) compared with normal subjects, indicating a beta-cell defect. Therefore, the aim of the present study was to investigate whether 4 weeks of near-normalization of blood glucose improves beta-cell responsiveness to GLP-1.
Materials and Methods: Nine obese T2DM in poor glycaemic control (HbA1c: 8.0 ± 0.4%, mean±SEM) were investigated before and after 4 weeks of near-normalization of blood glucose (mean BG: 6.4 ± 0.3 mmol/l by 7-points BG profile at least every third day) using insulin treatment. HbA1c after insulin treatment was 6.6 ± 0.3%. Nine matched control subjects were also studied. Before and after insulin treatment beta-cell function was investigated on two days with infusion of saline or GLP-1 (1.0 pmol/kg/min) during a stepwise graded glucose infusion at a rate of 2, 4, 6, 8 and 12 mg/kg/min. Each infusion rate was 30 min. The GLP-1 infusion resulted in a high physiological plasma GLP-1 concentration of approximately 80 pmol/l. The beta-cell responsiveness to glucose and glucose + GLP-1 was expressed as the slope of the linear regression between ISR and glucose concentration (pmol · min^{-1} · kg^{-1} · mmol^{-1} · L).
Results: The slope (mean±SEM) during the graded glucose + saline infusion were before and after insulin treatment: 0.33 ± 0.07 and 0.39 ± 0.04, p:NS. The corresponding slopes during glucose + GLP-1 infusion were 1.27 ± 0.2 and 1.56 ± 0.26, p:NS. GLP-1 improved beta-cell responsiveness significantly both before and after near-normalization of glucose with approximately a factor 3.8 and 4.0, respectively. For comparison the slope during the graded glucose + saline and glucose + GLP-1 infusion were 1.01 ± 0.14 and 4.79 ± 0.53, indicating an increase in beta-cell responsiveness with a factor 4.7 during GLP-1 infusion, p < 0.001.
Conclusion: 4 weeks of insulin treatment with near-normalization of blood glucose has no effect on beta-cell sensitivity to glucose or to GLP-1. Nevertheless, GLP-1 in physiological concentration restores beta-cell responsiveness to hyperglycaemia to a normal level.

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The suppression of postprandial glycaemia by glucagon-like peptide 1 in healthy subjects is closely related to slowing of gastric emptying
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Background and Aims: The mechanisms mediating the reduction in postprandial glycaemia by glucagon-like peptide 1 (GLP-1) are poorly defined. In healthy subjects, and patients with type 2 diabetes, exogenous administration of GLP-1 is associated with a reduction, rather than stimulation, of postprandial insulin secretion. We have evaluated the relationship between the effects of GLP-1 on glycaemia and gastric emptying.
Materials and Methods: 10 healthy men, age 27+/-2 yr, were each studied on three separate days, in double-blind, randomized fashion. Each subject received i.v. (1) GLP-1 (7–36 amide), 0.3 pmol/kg/min [0.3], (2) GLP-1, 0.9 pmol/kg/min [0.9], or (3) saline [C], commencing 30 min (i.e. t = -30 min) before ingestion of a radio-labelled mixed solid (100 g ^{99m}Tc-sulphur colloid minced beef) and liquid (150 mL ^{67}Ga-EDTA 10% dextrose) meal (total 270 kcal). Gastric emptying was measured using a scintigraphic technique for 120 min. For the solid (i.e. non carbohydrate) component, the intragastric retention at 100 min, and for the liquid (i.e. carbohydrate) component of the meal, the 50% emptying time (T50), were calculated. Blood glucose was measured every 15 minutes from t = -45 to 120 min. Data were evaluated using repeated measures ANOVA and linear regression. Results are shown (mean±SEM).
Results: GLP-1 [0.9] decreased preprandial blood glucose and both doses of GLP-1 markedly attenuated the postprandial rise in blood glucose. When compared with control, the blood glucose at 30 min was less after both doses of GLP-1 and lower after GLP-1 [0.9] than GLP-1 [0.3]. GLP-1 slowed gastric emptying of both solids and liquids without any significant difference between the two doses. There was a close relationship between the magnitude of the postprandial rise in blood glucose and gastric emptying of liquid (T50). The increase in blood glucose at t = 15 min was related to

the liquid T50 in the control (r = -0.73, p<0.01), GLP-1 [0.3] (r = -0.63, p<0.05), and GLP-1 [0.9] (r = -0.69, p<0.05) groups, as well as the total treatment group (r = -0.70, p<0.001) (i.e. the rise in blood glucose was greater when gastric emptying was relatively more rapid). The AUC for the change in blood glucose between 0–60 min was also related to the liquid T50 (r = -0.57, p< 0.001).

	Control [C]	GLP-1 [0.3]	GLP-1 [0.9]
Preprandial glucose (mmol/L)	5.4 ± 0.15	5.1 ± 0.15	4.9 ± 0.12*
Blood glucose at 30min (mmol/L)	7.4 ± 0.14	6.6 ± 0.31*	6.1 ± 0.27*¶
$AUC_{0-60\ min}$ blood glucose (mmol · min/L)	67 ± 7.2	35 ± 8.9*	41 ± 5.7*
Liquid gastric emptying (T50 min)	28 ± 2	42 ± 7*	50 ± 9*
Solid gastric emptying (% retention at 100 min)	28 ± 5	53 ± 6*	58 ± 7*

* p< 0.05 vs control ¶p<0.05 GLP-1 [0.3] vs GLP-1 [0.9]

Conclusions: In healthy subjects the dominant effect of exogenous GLP-1 on postprandial glycaemia is accounted for by slowing of gastric emptying.

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Tachyphylaxis is not the cause for reduced insulinotropic action of gastric inhibitory polypeptide (GIP) in type 2 diabetes

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Background and Aims: It is characteristic for type 2 diabetes that the quantitative impact of incretin stimulation of the endocrine pancreas is reduced or absent if compared to healthy control subjects because the insulinotropic activity of GIP is reduced or lost in persons with type 2 diabetes, while GLP-1 remains relatively active. Recent studies by Vilsbøll et al. (Diabetologia 2002; 45: 1111) showed a better insulinotropic response to GIP bolus injection than to a continuous infusion. The question arose whether this might be caused by tachyphylaxis. Therefore, we wanted to compare effects of GIP during repeated bolus injection and during continuous infusion in persons with type 2 diabetes, their first-degree relatives, and healthy control subjects.
Materials and Methods: Ten healthy controls (no first- or second-degree relatives with diabetes; 5 female/5 male; 57 ± 9 y.; BMI 30.4 ± 2.7 kg/m^2), 10 first-degree relatives of persons with type 2 diabetes (5 f./5 m.; 57 ± 8 y.; BMI 30,4 ± 5.1 kg/m^2), and 10 persons with type 2 diabetes (5 f./5 m.; 58 ± 6 y.; BMI 31.1 ± 6.0 kg/m^2) participated in two hyperglycaemic (150 mg/dl) clamp procedures from 0 to 210 min each, receiving in randomized order either two intravenous bolus injections of human synthetic GIP at 30 and 120 min (50 pmol/kg) or a continuous intravenous GIP infusion (2 pmol · kg^{-1} · min^{-1}) from 30 to 180 min. Glucose was measured every 5 minutes and insulin and C-peptide (ELISA) were determined in 5 to 30 minute intervals. Statistical evaluation was done using repeated-measures ANOVA.
Results: During continuous GIP infusion, the integrated increments in insulin and C-peptide were 4480 ± 1108 mU · l^{-1} · min and 452 ± 51 ng · ml^{-1} · min, respectively, in healthy controls. The same measurements were higher in first-degree relatives with 6681 ± 1500 mU · l^{-1} · min (n.s.) and 713 ± 81 ng · ml^{-1} · min (p < 0.05), and lower in type 2 diabetic subjects (1704 ± 456 mU · l^{-1} · min and of 252 ± 56 ng · ml^{-1} · min; both p < 0.05). GIP bolus injections caused rapid increments in insulin and C-peptide lasting approximately 60 min. Again, responses tended to be larger in first-degree relatives, and were reduced in type 2 diabetic subjects. There were no statistically significant differences between integrated incremental insulin secretory responses to the first and second GIP bolus in any of the groups.
Conclusion: Continuous and bolus administration of GIP in type 2 diabetes causes a similar impairment of insulinotropic effectiveness during hyperglycaemic (150 mg/dl) clamp experiments. In contrast to previous studies from our group there was no impairment but rather an increase of insulinotropic GIP action in first-degree relatives. There is no evidence for rapid (within hours) tachyphylaxis of the insulinotropic response to repeated GIP injections in type 2 diabetes or in first-degree relatives.

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Glucagon-like peptide-1, fused to human transferrin, is a long-acting and potent anti-hyperglycemic agent

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Background and Aims: We evaluated the effectiveness of a GLP-1 analog fused to human transferrin (hTf) as a potential agent to treat type 2 diabetes. GLP-1 itself has a short biological half-life and is therefore not suitable for routine treatment of type 2 diabetes. Non-glycosylated transferrin has a half-life of 14–17 days. It provides high bioavailability and biodistribution and should be stable in the circulation. Therefore, a GLP-1/hTf fusion protein should have a prolonged half-life.
Materials and Methods: Using recombinant technology, a DPP IV-resistant GLP-1 analog was fused to non-glycosylated human transferrin (hTf) in yeast. GLP-1/hTf was secreted into the simple growth defined yeast medium, free of human and animal-derived products. It was first tested in CHO/GLP-1R cells for its ability to activate GLP-1R - mediated adenylyl cyclase activation. It was next tested in non-diabetic and diabetic mice for its ability to; (1) decrease blood glucose, (2) increase insulin secretion, and, (3) increase beta cell proliferation. Plasma insulin was measured by ELISA (Crystal Chem) and blood glucose by glucometer (Bayer).
Results: GLP-1/hTf activated GLP-1R - mediated cAMP formation in CHO/GLP-1R cells (EC_{50} 2.0 nmol/l vs. 0.2 nmol/l for native GLP-1). Intraperitoneal (IP) GLP-1/hTf (1 and 10 mg.Kg) normalized blood glucose in diabetic mice (321 ± 36.5 vs. 114.5 ± 24 mg/dl: mean±SEM) by 4 hours after injection. Plasma insulin levels were also elevated 4 hours after injection (8.5 ± 0.5 vs. 10.8 ± 0.4 ng/ml: mean±SEM). In addition, glucose excursions were significantly lower in diabetic mice after a glucose tolerance test (GTT) when IP GLP-1/hTf (10 mg · Kg) had been administered 12 hours prior to the GTT. In non-diabetic mice, blood glucose was lowered by IP GLP-1/hTf for up to 36 hours. GLP-1/hTf had significant effects on islets of Langerhans. BrdU incorporation into nuclei of β-cells was increased as early as 24 hr after IP GLP-1/hTf, and by 72 hr BrdU incorporation had increased by 800%, compared to hTf treatment alone.
Conclusion: These results indicated that GLP-1/hTf is biologically active at the level of the GLP-1 receptor, that it can normalize blood glucose and increase insulin secretion in diabetic animals and that its activity is long-lived.

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Gastric inhibitory polypeptide receptors are presented in human subcutaneous adipose tissue: Effects of central obesity and weight loss

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Background and Aims: Gastric inhibitory polypeptide (GIP), a duodenal hormone, is released in response to feeding and produces a glucose-dependent stimulation of insulin secretion. Recent studies in rodents suggested that GIP directly links overnutrition to obesity. Despite evidence for GIP effects on fat metabolism in humans, the GIP-receptor (GIP-R) has not been identified in fat tissue. We identified GIP-R in human subcutaneous adipose tissue and tested the hypothesis that expression of this gene is influenced by obesity and weight loss.
Materials and Methods: The expression of GIP-R mRNA by real-time RT-PCR was measured in subcutaneous adipose tissue biopsies of 70 non-diabetic postmenopausal women. The effect of weight reduction was studied in 14 obese non-diabetic postmenopausal women.
Results: The GIP-R gene was highly expressed in human adipose tissue. Adipose GIP-R gene expression was negatively correlated with BMI, waist circumference, basal insulin and homeostasis model assessment index of insulin resistance ($HOMA_{IR}$) (p &lt 0.01 for BMI, p&lt 0.001 for $HOMA_{IR}$, waist circumference and insulin). Basal insulin concentration after adjustment for BMI and waist circumference correlated negatively with adipose GIP-R gene expression (p&lt 0.05). Adipose GIP-R gene expression was reduced in obese women with the highest waist circumference (p= 0.009 in Mann-Whitney-U test). Weight reduction did not change gene expression levels of GIP-R and basal insulin concentrations.
Conclusion: Our data suggest that decreased expression of the GIP-R gene is associated with signs of insulin resistance in obese women. Increased basal insulin concentrations influence negatively the expression of the GIP-R gene in adipose tissue. Thus insulin is possibly a regulator of GIP-R gene expression in adypocytes. The absence of a increase of GIP-R gene expression after weight loss may be due to the fact the insulin levels also were not

decreased. This suggest that GIP-R antagonist may be promising in the treatment of obesity.

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Differential GIP and GLP-1 release following pure protein ingestion in mice

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Background and Aims: The incretins glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are major mediators of prandial insulin release after glucose and mixed meal ingestion, although their differential role following ingestion of the major macronutrients remains unknown. In this study we have compared the responses of both active and total levels of GIP and GLP-1 after oral administration of glucose versus pure protein in mice.
Materials and Methods: Isocaloric amounts of glucose (75 mg) and pure whey protein (WP, 75 mg), alone and in combination, were administered by gastric gavage, together with acetaminophen (AAP, 100 mg/kg), to overnight fasted anaesthetized female C57BL/6J mice. Controls received saline containing AAP. Oral glucose tolerance, glucose-stimulated insulinemia and gastric emptying were assessed by calculating the area under the curve for plasma glucose, insulin and AAP values, respectively, between t=0 and t=120 min. Blood for the assessment of incretin release was collected in tubes containing the DPP-4 inhibitor valine-pyrrolidide (0.01 mmol/L, final concentration in blood). Total GIP and GLP-1 were measured by RIA using C-terminally directed antisera, which react with intact peptides and N-terminally truncated metabolites. Intact GIP and GLP-1 were measured using N-terminally directed antisera.
Results: When given together with glucose, WP enhanced glucose tolerance resulting in 31% lower plasma glucose levels ($P<0.005$ vs. glucose alone). In spite of the lower glucose concentrations, insulin responses to glucose were markedly (3-fold) augmented by WP ($P<0.0001$ vs. glucose alone). WP markedly potentiated glucose-mediated GLP-1-release ($P<0.001$), which was associated with increased levels of intact bioactive GLP-1 ($P<0.01$). WP also raised intact GIP ($P<0.01$ vs. glucose alone), although total GIP was not augmented. Gastric emptying, as judged by plasma AAP, was reduced by 28% by glucose ($P<0.05$ vs. controls) with a further reduction when glucose was combined with WP (59%, $P<0.05$ vs. glucose alone).
Conclusion: The data indicate that pure protein ingestion augments active GLP-1 by stimulating GLP-1 release whereas active GIP is induced by reducing GIP inactivation. Therefore, in spite of reducing gastric emptying, WP enhanced circulating levels of intact GLP-1 and GIP in association with marked augmentation of insulin release and improved glucose tolerance. We therefore suggest that both GIP and GLP-1 are of importance for the augmented insulin response to oral protein ingestion.

	Total GLP-1	Intact GLP-1	Total GIP	Intact GIP	n
Glucose	59.9 ± 2.5	8.1 ± 0.4	593.6 ± 17.8	72.4 ± 3,2	21
WP	33.8 ± 1.9***	7.0 ± 0.3	62.8 ± 4.1***	63.6 ± 3,7	11
Glucose+WP	106.8 ± 10.5***	15.8 ± 4.0**	621.9 ± 9.2	91.7 ± 5,8**	10

Mean plasma incretin concentrations (pmol/L)±SEM 15 min after oral administration of macronutrients. **P<0.01 and ***P<0.001 vs. glucose group.

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Selective upregulation of the beta-cell specific Zinc-Transporter 8 (ZnT-8) by GLP-1 in INS-1E cells

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Background and Aims: Beta-cells of the pancreas accumulate large amounts of zinc. Apart from its role in metallo-enzymes, zinc is essential for the storage of insulin in secretory granules of beta-cells. Zinc binds to insulin in a 2 Zn - 6 proinsulin/insulin compound that is dissolved and released in states of hyperglycaemia. Zinc that is co-secreted with insulin has its own role in regulating beta-cell mass and glucagon secretion. Zinc deficiency impairs glucose-induced insulin secretion. Zinc is transported into cells and into specialized compartments of cells by specific zinc transporters (ZnT-1-8). ZnTs are differentially expressed in different tissues and have varying functions and distributions in different cell types. Recently, ZnT-8 was found to be specifically expressed in beta-cells. ZnT-8 is localised at the membrane of insulin-containing granules indicating that ZnT-8 is important for zinc transport into granules. Since zinc transports is of vast importance for insulin storage and secretion, we here describe the expression of different ZnTs in beta-cells.
Materials and Methods: We stimulated pancreatic beta-cells of rat origin, INS-1E cells, with GLP-1 and DEDTC, a zinc chelator, for 24 hours and investigated the expression-profile of ZnT-1-8 by Real Time PCR and normalisation to the housekeeping gene Cyclophilin A.
Results: We found an increased expression of ZnT-8 after GLP-1 stimulation ($P<0.05$) (figure 1). This stimulation of expression was restricted to ZnT-8 since the expression of ZnT-1-ZnT-7 did not change during GLP-1 stimulation. Furthermore, we found that DEDTC increased the expression of ZnT-3 2-fold ($P<0.05$) (figure 1). DEDTC stimulation did not affect the expression of ZnT-1, ZnT-4, ZnT-5, ZnT-6, ZnT-7 or ZnT-8. Finally, we found that ZnT-2 was not expressed in INS-1E cells.
Conclusions: We found that expression of vesicle-localised ZnT-8 can be manipulated by GLP-1. GLP-1 stimulates insulin synthesis and secretion. Our data imply that an increased need for zinc during storage in secretory vesicles is met by an increase of ZnT-8 expression since no other ZnTs were regulated by GLP-1 stimulation. The expression of ZnT-3 has not previously been described in beta-cells but ZnT-3 has been found in cerebral synaptic vesicles possibly being responsible for zinc recycling. We show here that zinc chelating by DEDTC seems to increase expression of ZnT-3 in INS-1E cells.

Figure 1. Expression ratio between ZnTs and Cyclophilin A. *P<0.05

DEDTC	GLP-1	Control	
ZnT-8	1.682 (SD=+/-1.22)	1.653 (SD=+/-0.45) *	1.163 (SD=+/-0.33)
ZnT-3	2.522 (SD=+/-1.36) *	1.489 (SD=+/-1.06)	1.172 (SD=+/-0.32)

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Cyclic activation of osteoblasts by GIP as an endogeneous osteogenic factor

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Background and Aims: Diabetes is associated with increased risk of osteoporosis, although little is knouwn about the cause. Gastric inhibitory polypeptide/glucose-dependent insulinotropic polypeptide (GIP) plays not only as an incretin but also as a factor that mediates nutrient accumulation in adipocytes. GIP receptor (GIPR) is widely expressed in various cells including pancreatic beta-cells, adipocytes and osteoblasts. In this study, we investigated the role of GIP on pathophysiology of diabetic osteopathy using GIPR knockout (*Gipr*$^{-/-}$) mice.
Materials and Methods: 8-week-old male *Gipr*$^{-/-}$ mice were used for histochemical analysis. Osteoclastic function was examined by pit-forming activity of crude osteoclast preparations placed on dentine slices. Saos-2 cells (human osteoblastic cell-line) were used for Etoposide-induced apoptosis assay.
Results: First of all, we confirmed the expression of GIPR and parathyroid hormone (PTH) receptor in mouse islets, kidney, liver, jejunum, and MC3T3E1 osteoblastic cells by RT-PCR method. *Gipr*$^{-/-}$ mice had similar naso-anal length to wild-type (*Gipr*$^{+/+}$) mice and soft X-ray analysis showed that longitudinal growth of limb bones was not affected in *Gipr*$^{-/-}$ mice, which indicates that GIP signaling does not affect endochondral ossification. We then found that the bone trabeculae of *Gipr*$^{-/-}$ mice were thinner in histology than those of wild-type mice, which is compatible for osteoporosis. Moreover, postprandial plasma calcium level and urinary deoxypyridinoline concentration were increased in *Gipr*$^{-/-}$ mice. Based on these findings, we hypothesized that GIP, especially postprandial high level of GIP, might have some effects on dietary calcium dynamycs, in particular osteogenesis. We performed alkaline-phosphatase immunostaining for osteoblasts and tartrate-resistant acid-phosphatase staining for osteoclasts, and revealed that the number of osteoclasts were increased in *Gipr*$^{-/-}$ mice, while that of osteoblasts was unchanged. As GIP had no direct effect on

osteoclasts, we focused on the effects of GIP on osteoblasts. GIPR mRNA was expressed in Saos-2 osteoblastic cells and GIP stimulated cAMP production in these cells, which means Saos-2 cells have functional GIPR. We demontrated that pre-treatment of GIPsignificantly reduced Etoposide-induced apoptosis in Saos-2 cells, like intermittent administration of PTH, which has been reported to prevent osteoporosis by protecting osteoblasts from apoptosis.
Conclusion: Cyclic activation of cell surface receptors often leads to a different biological response than sustained activation. The differential responses of skeletal bone to intermittent elevation of endogeneous GIP versus continuous elevation of endogeneous PTH, both of which increase the intracellular cAMP concentration, represent a systemic regulator with important clinical and therapeutic implications. We propose that GIP plays a role in coordinating nutrient utilization for bone formation through proliferative effects on osteoblasts and the decreased GIP signal is one of the causes of the diabetic osteopathy.
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PS 38
Rodent models of diabetes

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Gene dose effects of the leptin gene
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Background and aims: The hyperglycemic and grossly overweight ob/ob mouse lacks functional leptin. Heterozygous mice (ob/+) have a largely normal phenotype. We have now compared ob/+ and +/+ mice with regard to metabolic variables and sensitivity to diet change. A gene dose effect of leptin could be important because there are many individuals with low normal leptin levels.
Materials and Methods: Phenotypically 'lean' mice from a local colony (Umea; ob/ob) were identified as +/+ or ob/+ at the age of three weeks. The Lepob mutation was detected with PCR using the primers OBW-1, OBW-2 and OBM-1. Weight increase, blood glucose levels, and food intake were measured. Glucose tolerance tests were performed. The mice were observed for 18 months. Normal mouse diet contained 5% saturated fat and 1260 kJ/100 g. A high carbohydrate diet (70% carbohydrate) or a high fat diet (21% fat, 1560 kJ/100 g) were introduced at 9 and 16 months, respectively.
Results: At 3 weeks ob/+ mice weighed 13.5 ± 0.2 g (N=13) and +/+ weighed 12.3 ± 0.3 g (N=9, P<0.01). Ob/+ mice increased weight particularly between the ages 16 and 28 weeks. The difference obtained (5.1 g compared with +/+, P<0.05) is maintained troughout the observation period. Blood glucose levels were higher in 3 w old ob/+ mice (8.2 ± 0.4 vs. 6.9 ± 0.2 mmol/l, P<0.05) but that difference disappeared after 3 m. Ob/+ mice had a slightly higher food intake (25.0 vs. 22.1 g/animal/w). All animals increased energy intake and weight when given a high-fat diet. Food intake was slightly reduced during the high-carbohydrate diet but body weight was not changed. There was no difference in weight development between ob/+ and +/+ during 8 w with high-fat or high-carbohydrate diet, and also no difference in i.p. glucose tolerance tests. However, during high-carbohydrate feeding blood glucose decreased –0.5 ± 0.6 mmol/l in +/+ mice and increased 1.0 ± 0.4 mmol/l in ob/+ mice (P=0.05).
Conclusion: There is a difference between ob/+ and +/+ animals with regard to body weight increase at a young age when the animals grow rapidly. Ob/+ animals may be more sensitive to a high-carbohydrate diet. A gene dose effect of the leptin gene can have important implications for the development of obesity and type 2 diabetes.

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Altered substrate utilization caused by respiratory uncoupling in white fat of aP2-*Ucp1* transgenic mice
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Background and Aims: Increased expression of uncoupling proteins (UCPs) in adipose tissue is associated with the conversion of lipid-storing white adipocytes into lipid-oxidizing cells, suggesting an efficient strategy for the treatment of obesity and metabolic syndrome. However, the metabolic consequences of such a conversion need to be precisely evaluated. Therefore, we analyzed substrate fluxes in the transgenic mice resistant to obesity due to fat-specific overexpression of UCP1 gene.
Materials and Methods: Hemizygous and homozygous transgenic aP2-*Ucp1* mice and their non-transgenic littermates (C57BL/6J strain, males) were maintained at 22°C and fed *ad libitum* a chow diet or exposed to a high fat diet for 8 weeks. When indicated, animals were fasted overnight (~16 hours). Intraperitoneal glucose and pyruvate tolerance tests were used to assess glucose tolerance and gluconeogenesis, respectively. Whole-body oxygen consumption and deep body temperature were analyzed by indirect calorimetry and telemetry (system INCA, Somedic, Sweden), respectively. Gene expression was assessed by real-time quantitative RT-PCR.
Results: Fasted blood glucose levels in the hemizygous transgenic mice were ~30% higher ($P < 0.001$) than in non-transgenic mice despite normal insulin levels. In agreement with increased hepatic gluconeogenesis, glucose levels during pyruvate tolerance test were significantly higher in the transgenic mice under the setting of normal glucose tolerance. Conversely, plasma NEFA and triglycerides were proportionately decreased in the transgenics ($P < 0.001$). During fasting, whole body oxygen consumption (controls 55.5 ± 3.0 vs. transgenics 45.8 ± 0.7 ml/kg/min, $P < 0.05$) and deep body temperature (controls 35.1 ± 0.3°C vs. transgenics 33.8 ± 0.3°C, *P*

<0.05) were lower in transgenic than in control mice, suggesting possible defect in brown fat thermogenesis induced by ectopic UCP1. When exposed to a high-fat diet, transgenic mice showed a tendency to maintain elevated blood glucose in the fasted state (controls 139 ± 7, hemizygous transgenics 150 ± 5, homozygous transgenics 167 ± 6 mg/dL; P <0.05 controls vs. homozygous transgenics). Gene expression analysis confirmed up-regulation of gluconeogenic enzymes PEPCK and glucose 6-phosphatase in the liver of transgenic mice by ~60% (P < 0.01) and ~120% (P < 0.001), respectively. Plasma insulin was proportionately decreased by the trangene dosage (controls 0.308 ± 0.080, hemizygous transgenics 0.182 ± 0.019, homozygous transgenics 0.105 ± 0.024 ng/mL; P <0.05 controls vs. homozygous transgenics) despite reduced expression of *Glut4* in adipose tissue of transgenic mice (P < 0.001). High-fat diet potentiated the effect of transgene on plasma triglyceride levels that were decreased by ~50% (P <0.001) in the hemizygous transgenic mice.
Conclusion: Overexpression of UCP1 in adipose tissue is associated with increased gluconeogenesis in mice fed both standard chow and high-fat diets. This suggests a stimulation of sympathetic nervous system compensating for the relative insufficiency of brown fat thermogenesis in transgenic mice rather than a consequence of insulin resistance, since insulin levels in transgenic mice remain low. The mechanism by which overexpression of UCP1 in white fat sensitizes to insulin remains to be established.
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The role of adipose tissue and adipose tissue-derived factors in the development of type 2 diabetes. Studies in a new type 2 diabetes animal model lacking phosphodiesterase 3B
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Background and Aims: A variety of proteins secreted from the adipose tissue, adipokines, are thought to be of importance in the connection between obesity and type 2 diabetes. cAMP is a critical second messenger generating different biological responses. Important in cAMP signaling is degradation of cAMP by the cyclic nucleotide phosphodiesterases (PDEs). PDE3B is expressed in tissues of importance for energy homeostasis, such as pancreatic β-cells, hepatocytes, and adipocytes. In the β-cell, raised cAMP promotes insulin synthesis and secretion. PDE3B knockout (KO) mice show defects in energy homeostasis and insulin resistance. In vivo, PDE3B KO mice have been shown an altered release from adipose tissue in response to beta-adrenergic agonists, which caused an increase insulin secretion. An increased metabolic and/or uncoupling activity has also been observed in KO mice compared to wildtype (WT) mice. PDE3B KO mice show resistance to weight gain on high fat diet, have less body fat and smaller adipocytes. Both these observations seen in vivo have now been studied in vitro for evaluation of the molecular mechanisms. The aim of this project is to find the molecular factor(s) involved in the cross-talk between adipocytes and pancreatic β-cells with special interest in cAMP and cAMP-regulated proteins, and to further examine the increased metabolic and/or uncoupling activity observed in PDE3B KO mice.
Materials and Methods: White and brown adipose tissues and islets of Langerhans were collected from PDE3B deficient mice. Tissues from PDE3B wildtype (SvJ129) mice were used as control.
Results: Isolated primary adipocytes from epididymal fat pads from PDE3B KO and WT mice were stimulated with the β3-specific adrenergic agonist CL 316,243. The subsequent adipocyte medium was used to stimulate the islets of Langerhans. Insulin secretion was shown to be more pronounced in islets treated with CL-stimulated adipocyte medium and this effect was even more pronounced in the PDE KO mice. The observed difference between KO and WT mice was not due to increased lipolysis since there were no observed difference in glycerol release. Since β3-adrenergic receptors are predominantly expressed in adipose tissue and are not present in pancreatic β-cells, the observed effect of increased insulin secretion is suggested to be caused by increased production/release of some adipokine in response to CL in KO adipose tissue, or/and increased responsiveness to this signals in β-cells of the KO mice. Oxygen consumption measurements in vitro in both white and brown adipose tissue shown a significant increase in PDE3B KO mice compared to WT mice.
Conclusion: It is suggested that the increased insulin secretion observed as an effect of β3-specific agonists is generated via signals derived from the adipose tissue. In the PDE3B deficient mouse model, adipocyte medium from adipocytes treated with the β3-specific agonist CL seems to contain increased concentrations of this not yet identified adipokine(s) that is responsible for the enhanced insulin secretion. The PDE3B deficient mice have also shown an increased oxygen consumption in both white and brown adipose tissue in vitro compared to WT.

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β-cell specific hormone-sensitive lipase knock out mice exhibit normoglycemia and intact insulin secretion
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Background and Aims: Hormone sensitive lipase (HSL) is expressed predominantly in adipose tissue, where it is believed to play an important role in lipolysis of stored triglycerides. The enzyme is also expressed in pancreatic β-cells. HSL is activated by catecholamines and other lipolytic hormones, and inactivated by insulin. These features and the potential role of lipids in regulation of insulin secretion as well as their toxic effects on β-cell function, have prompted analysis in several laboratories of HSL knock out mouse lines with regard to β-cell function. However, the results from these analyses have not provided a clear picture of the role of HSL in β-cells. This could be attributed to genetic redundancy effects, different genetic background or physiological compensatory mechanisms. With this in mind, we have created a β-cell specific knock out of HSL in hope to better understand the role of HSL in β-cells.
Materials and Methods: Through cross breeding of a mouse line [SV129/C57BL6J] carrying a mutated HSL allele flanked by lox p sites with a mouse line [C57BL/6J] expressing the cre recombinase under control of the rat insulin 2 promoter mice with a deletion of the HSL gene specifically in β-cells were created (β-HSL KO).
Results: Using RT-PCR, the truncated HSL transcript was detected in β-HSL KO islets at 5 weeks of age; wild type (WT) islets expressed only the full length HSL transcript. This indicates that recombination had efficiently occurred in β-cells. The mice exhibited no difference in body weight up until 18 weeks of age on a normal diet. Fed plasma glucose levels were similar in the two genotypes up until 18 weeks of age; at 9 weeks, plasma glucose was 11.78 ± 1.49 in male and female 11.49 ± 0.5 β-HSL KO mice compared to male and female 9.77 ± 2.0 - 10.85 ± 0.8 WT mice. Insulin secretion in batch incubations of freshly isolated islets was similar in the two genotypes at 9 weeks. Furthermore, insulin secretion in response to palmitate, GLP-1 etc was also unchanged in the β-HSL KO mice.
Conclusion: At this point, our studies of a β-cell specific knock out of HSL have not revealed any clear phenotypic differences that can be attributed to presence or absence of HSL. Thus, these studies in a β-cell-specific KO of HSL, which should at least in part circumvent problems inherent in genetic background, redundancy and compensatory effects, do not support an important regulatory role of HSL in β-cells. This is in agreement with the results from previous studies of a general HSL knock out created in our laboratory. However, the possibility remains that the recombination has not been efficient enough to abolish all actions of HSL in β-cells, which may be more permissive in nature. Our future studies will address these issues.
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Inducible LIRKO shows insulin resistance and glucose intolerance without liver dysfunction
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Background and aims: Liver is the major tissue involved in the regulation of glucose homeostasis. To assess the importance of insulin receptor in the liver-mediated glucose homeostasis after liver maturation was accomplished, we have generated liver-specific insulin receptor knockout mice in an inducible-manner (iLIRKO).
Materials and Methods: We crossed animals with the exon 4 from insulin receptor flanked by LoxP sequences (LoxP/LoxP-IR mice) and Mx-Cre mice, bearing recombinase enzyme driven by Mx-promoter. This promoter was induced by double-strand RNA (pI-pC). pI-pC was intraperitoneally injected three times in alternative days for 1 week in 21 days-old mice. Then, alternatively insulin tolerance or glucose tolerance tests were performed up to 18 weeks. Afterwards, mice were sacrificed and liver, kidney, heart, muscle, brown adipose tissue and white adipose tissue were collected. Tissue extracts were used to test insulin receptor expression levels and also the expression of several enzymes related with glucose and lipid metabolism by Western-blot.

Results: iLIRKO mice showed a severe insulin resistance in all animals studied. However, onset of insulin resistance changed individually, insulin resistance achieved being irreversible. iLIRKO mice showing insulin resistance developed a severe glucose intolerance, that was ameliorated or even reverted depending on the group of animals studied. Insulin receptor was deleted by 90%. No effect was observed on other major insulin target tissues studied. iLIRKO males had a manifest hyperinsulinemia (around 6 +/– 1.5 ng/ml). Concurrently, glucokinase expression was completely blunted in iLIRKO compared with LoxP/LoxP-IR male mice. However, no effect was observed on PEPCK, or FAS, or Glut-2 protein expression.
Conclusions: Our results indicate that iLIRKO mice give rise of sustained insulin resistance that course with severe glucose intolerance. This phenotype was associated with massive insulin receptor and glucokinase liver-specific depletion. Furthermore, these animals showed manifest hyperinsulinemia associated with beta-cell hyperplasia that may compensate glucose intolerance upon time.
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Farnesoid X receptor (FXR) regulates peripheral insulin sensitivity
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Background and Aims: The farnesoid X receptor (FXR) is a bile acid (BA)-activated nuclear receptor that plays a major role in regulating BA and lipid metabolism. More recently, several studies have suggested a potential role of FXR in glucose metabolism. Indeed, hepatic FXR expression is reduced in animal models of diabetes. However, the exact contribution of FXR in the control of glucose homeostasis remains to be determined. Here, we investigated whether FXR-deficiency could alter insulin sensitivity in vivo in mice.
Materials and Methods: Experiments were performed on homozygous male FXR-deficient (FXR-/-) mice and wild type mice (FXR+/+) bred on the C57BL/6N genetic background, and on ob/ob mice and their lean littermates. Intra-peritoneal glucose tolerance test (IPGTT) and insulin tolerance test (ITT) were performed after 6 h of fasting. Hyperinsulinemic-euglycemic clamps were performed in chronically catheterised freely moving FXR-/- and FXR+/+mice after 9 h of fasting. For in vivo analysis of insulin signaling, mice were starved during 24 h, and injected with 0.1 IU/kg of human insulin into the portal vein. Tissues were removed 5 min after injection and frozen in liquid nitrogen until western blot analysis. In vivo treatment with non-steroidal specific FXR agonist GW4064 (30 mg/kg/day) was given intra-peritoneally during 8 days.
Results: In basal conditions (after 6 h of fasting), blood glucose levels were significantly decreased in FXR-/- mice compared to controls, linked to an impaired fasting response in these animals. Plasma insulin levels were also decreased in FXR-/- mice, likely reflecting an adaptive response to the relative hypoglycaemia. In contrast, FXR-/- mice had an increased glucose excursion during IPGTT, indicating a glucose intolerance. Furthermore, FXR-/- mice exhibited less reduction in glucose levels during an ITT (0.75 IU/kg) than control mice. To definitively assess the changes in insulin sensitivity in FXR-/- mice, a hyperinsulinemic-euglycemic clamp study was performed. Glucose infusion rate was decreased in FXR-/- mice, demonstrating a whole-body insulin resistance in these mice. While hepatic glucose production rate did not differ between both strains, whole-body glucose uptake was decreased in FXR-/- mice, indicating a peripheral insulin resistance. To determine whether FXR-deficiency altered insulin signaling, we analyzed the level of insulin-stimulated Akt (PKB) phosphorylation in both white adipose tissue and skeletal muscle. Interestingly, an approximate 50% decrease in the insulin-dependent Akt phosphorylation on serine 473 was observed in both peripheral insulin sensitive tissues of FXR-/- mice. Finally, we investigated whether an in vivo treatment with a specific FXR synthetic agonist (GW4064) could improve the insulin resistance seen in genetically ob/ob mice. While blood glucose levels did not change, plasma insulin levels were significantly reduced after 8 days of GW4064 treatment. The ability of FXR agonist to increase insulin sensitivity was further established in ITT. Indeed, the same insulin dose (2 IU/kg) caused a more pronounced decrease in blood glucose levels when given to GW4064-treated ob/ob mice.
Conclusion: These results provide new evidences for a role of FXR in the regulation of insulin sensitivity. This unexpected function of FXR opens attractive perspectives for the treatment of type 2 diabetes.

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Impaired adaptive response to fasting in FXR-deficient mice
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Background and Aims: The farnesoid X receptor (FXR) is a bile acid-activated nuclear receptor. Recently, several studies have suggested that FXR might regulate carbohydrate metabolism. Hepatic FXR expression varies during nutritional changes in mouse liver, with an increased expression during fasting. Here, FXR-deficient mice were subjected to long-term fasting protocol to investigate the role of FXR *in vivo* under such a physiological condition.
Materials and Methods: Homozygous FXR-deficient ($FXR^{-/-}$) mice and wild type mice ($FXR^{+/+}$) bred on the C57BL/6N genetic background were fasted during 48 h. Tail vein blood samples were taken for blood glucose measurements. Eye blood samples were taken at 0 h, 6 h, 24 h and 48 h for plasma metabolites measurements. Mice were sacrificed at 6 h or 48 h and liver was removed for RNA extraction and real time quantitative RT-PCR analysis. Hepatic glucose production (HGP) rate was calculated in chronically catheterised freely moving $FXR^{-/-}$ and $FXR^{+/+}$mice by isotope dilution after 9 h of fasting. Hepatocytes were isolated from the livers of fed $FXR^{-/-}$ and $FXR^{+/+}$ mice by a modification of the collagenase method.
Results: While there was no difference in the fed state, after fasting blood glucose levels dropped more rapidly in $FXR^{-/-}$ than in control mice, with values statistically lower at 3 h (122 ± 4 *vs.* 153 ± 4 mg/dl; $p<0.001$), 6 h (121 ± 3 *vs* 135 ± 3 mg/dl; $p<0.01$) and 12 h (135 ± 7 *vs* 161 ± 7 mg/dl; $p=0.04$). In contrast, blood glucose levels did not differ after 24 h and 48 h of fasting between both strains. Blood insulin levels followed glycaemia with lower concentrations after 6 h of fasting in $FXR^{-/-}$ mice. On the other hand, no differences were found in plasma β-hydroxybutyrate and free fatty acid (FFA) concentrations between both strains, suggesting that FXR-deficiency did not alter hepatic FFA oxidation. The expression of genes encoding various enzymes and transcription factors involved in the regulation of HGP was measured. The expression of phosphoenolpyruvate carboxykinase (PEPCK) mRNA, a key-enzyme of gluconeogenesis, was significantly decreased by 59% ($p<0.001$) in liver of $FXR^{-/-}$ mice compared to controls. In contrast, mRNA expression of glucose-6-phosphatase, fructose 1, 6-bis phosphatase, and the coactivator PGC-1α did not differ between both strains. Then, PEPCK mRNA levels in isolated hepatocytes from $FXR^{-/-}$ and $FXR^{+/+}$ mice were measured. FXR-deficiency led to a 66% decrease ($p<0.01$) in basal PEPCK mRNA expression compared to controls, confirming that FXR directly regulates PEPCK gene expression in mouse hepatocytes. Of functional relevance, endogenous glucose production was significantly lower in $FXR^{-/-}$ mice than in controls (103 *vs.* 136 µmol/kg/min; $p<0.05$), suggesting that the decreased PEPCK expression observed in liver from $FXR^{-/-}$ mice may contribute to an impaired gluconeogenesis during fasting. Since hypoglycaemia occurred within 3 h after food withdrawal, we also investigated whether glycogenolysis could be altered in $FXR^{-/-}$ mice. Interestingly, basal (post-absorptive) hepatic glycogen content was decreased by 54% in $FXR^{-/-}$ mice compared to controls.
Conclusion: FXR regulates the kinetics of metabolic changes during short-term fasting. Thus, FXR appears to play an unexpected role in control of fuel availability, a function that opens exciting perspectives for the treatment of metabolic disorders, such as type 2 diabetes and obesity.

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Modeling metabolic syndrome in complex genetic systems
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Background: Obesity, insulin resistance and dyslipidemia are interrelated traits that characterize metabolic syndrome. In an effort to understand the genetics that connect these traits we used Multigenic Obesity (MOB) loci 5 and 6 congenic mice to partition genomic intervals previously shown to contribute to metabolic syndrome and which were syntenic to human loci linked to NIDDM 3, Bardet Biedel Syndrome and Familial Combined Hyperlipidemia. Here, we introgressed metabolic syndrome trait resistant Casteneus (CAST) alleles from chromosome 2 onto a C57BL6 (B6) metabolic syndrome trait susceptible background to create 2 congenic strains. We hypothesized that unique CAST alleles from an interval 72–84 Mb contributed to the decreased adiposity seen in the MOB 6 congenics while pre-

disposing them to the dyslipidemia associated with atherosclerosis. But CAST alleles unique to the MOB 5 locus from 162–180.2 Mb contributed to the insulin resistance observed in this strain. And while CAST intervals unique to each MOB congenic restricts the number of genetic influences that contribute to their phenotypic differences, the size of each unique locus was too large to identify the gene/s that mitigate the obesity but aggravate dyslipidemia in the MOB 6 congenics and exacerbate the insulin resistance in the MOB 5 congenics.
Methods: To ehance the affect of MOB CAST alleles on a metabolic syndrome phenotype with cardiovascular complications each MOB strain was bred onto a B6 - LDL receptor null (L-/-) background and fed a Western diet. To test our previous hypotheses each MOB locus was restricted to it's unique genomic interval by backcrossing to create MOB 5 and 6 sub-congenic strains. All MOB derived strains were characterized and compared to control strains for plasma lipid content, fat mass accretion by NMR, insulin resistance by IPGTT and food efficiency. L-/- MOB congenics were also assayed for atherogenesis by en face analysis of the entire aorta. We used Affymetix's MG_U74v2 chip set to profile expression changes in 36,000 genes from liver, muscle and adipose to identify candidate genes that elicit the phenotypic differences observed between MOB congenics and B6 controls.
Results: In an environment of nutritional excess, MOB 6 derived congenics were resistant to the atherosclerosis, obesity and insulin resistance typical of L-/- and B6 controls but the MOB 5 derived congenics showed decreased insulin sensitivity, increased fat mass accretion and dyslipidemia. Comparison of gene expression profiles between B6 controls and MOB congenics revealed a marked increase in expression changes in adipose compared to liver and muscle. These data suggest that alterations in MAPK signaling through NF kappa B in adipose underlie the obesity, and insulin resistance of the MOB 5 congenics. But arrray data confirmed in vivo by NMR analysis of Pallid null mice identified a novel candidate gene for diet induced obesity.
Conclusions: Congenic mouse strains are complex genetic systems that replicate the intricate interplay between syntenic human loci to provide functional models, valuable tools, for the identification of genes and metabolic pathways that underlie complex human disorders.
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Reduced glucose stimulated insulin secretion in the Cohen diabetic sensitive rat is associated with abnormal fat deposit
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Background: Ectopic fat deposit is a major cause for β cell dysfunction and insulin peripheral resistance. The Cohen diabetic sensitive (CDs) rats when fed diabetogenic high sucrose diet (HSD) develops type 2 diabetes characterized by reduced glucose stimulated insulin secretion (GSIS) and abnormal pancreatic morphology. Conversely, the Cohen diabetic resistant (CDr) rat maintains normoglycemia with preserved pancreatic morphology when fed HSD.
Aim: to examine the relationship between fat accumulation in the pancreas or muscle and the mode of manifestation of type 2 diabetes in the CDs fed HSD.
Methods: 6 males CDs and CDr rats were fed HSD for 1 month (CDs-HSD and CDr-HSD respectively). Blood glucose levels were assessed after overnight fast and during oral glucose tolerance test (OGTT, 3.5 g/kg) and reassessed following insulin tolerance test (ITT 0.1U/kg). Blood triglycerides (TG) and free fatty acid (FFA) levels were measured at fasting and postprandial states. At termination, rats were sacrificed, the pancreas and gastrocnemius muscle were removed and weighed. Fatty infiltration was assessed by oil red O and osmium staining using light and electron microscopy. TG content was assessed from total fat extracted by chloroform-methanol (Folch).
Results: The area under the OGTT curve was significantly elevated in CDs-HSD compared to CDr-HSD (1750 ± 123 vs. 836 ± 38 mM P<0.001). Despite the high glucose levels, insulin secretion was significantly lower in CDs-HSD compared to CDr-HSD (AUC 24186 ± 4508 vs 64397 ± 9111 pM, P=0.004). ITT revealed similar sensitivities of the peripheral tissues of CDs-HSD and CDr-HSD rats to exogenous insulin. Levels of FFA were significantly higher in CDs-HSD compared to CDr-HSD (1.484 ± 0.12 vs. 1.064 ± 0.06 mM, P = 0.04) while TG were not different. In postprandial state, glucose levels of the CDs-HSD were significantly higher compared to CDr-HSD (16.3 ± 1.2 vs. 6.2 ± 0.3 mM, P< 0.001) with no difference in TG and FFA levels. Histological evaluation of the pancreas of CDs-HSD showed atrophy of the exocrine acinar tissue with fatty infiltration but preservation of islet morphology and insulin content. Fatty infiltration was confined to the exocrine tissue while no fat was found in the gastrocnemius muscle. TG content of the pancreas was significantly higher in CDs-HSD compared to CDr-HSD (5.2 ± 0.92 vs. 1.2 ± 0.21 mg/g tissue p<0.002). Pancreatic weight was significantly lower in CDs-HSD compared to CDr-HSD (0.17 ± 0.02 vs. 0.56 ± 0.05 g, p<0.05). The CDr-HSD exhibited normal pancreatic morphology and no fatty infiltration in pancreas and muscle.
Conclusion: Our data shows an association between fat accumulating in the exocrine pancreas but not in the muscle, with reduced GSIS and preserved sensitivity of peripheral tissues to insulin. We hypothesize that in animal as well as in man there is a strong association between the organ target of ectopic fat accumulation and its outcome being peripheral insulin resistance or β-cell dysfunction.
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Reduced glucose stimulated insulin secretion is associated with progressive exocrine pancreatic lesions in the Cohen diabetic sensitive rat
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Background: Recent studies show that impairment of exocrine function and pancreas morphology is more frequent in patients with type 2 diabetes then estimated in the past. The Cohen Diabetic sensitive (CDs) rat fed a high-sucrose copper poor diet (HSD) develops type 2 diabetes accompanied by dysmorphogenesis of the exocrine pancreas, decreased glucose stimulated insulin secretion (GSIS) with no peripheral insulin resistance.
Aims: To define a cause-effect relationship between the progressive changes in the exocrine morphogenesis and β-cell dysfunction isolating the effects of time period on HSD and the copper level in the diet. To compare the insulin output from the isolated perfused pancreas to that obtained in vivo by intravenous tolerance test (IVGTT).
Methods: 5 males CDs rats were fed HSD-copper-poor diet for 10, 20 and 30 days or HSD supplemented with adequate concentration of copper (HSD-Cu) for 30 days. Serum insulin and glucose level were measured after overnight fast and during IVGTT (7.5 mg/kg BW) in rats fed HSD containing low or adequate levels of copper. At termination, the pancreas was removed, weight and evaluated for apoptosis (TUNEL), macrophage and fatty infiltration (oil red O). Insulin secretion was assessed in perfusate of CDs fed HSD.
Results: In IVGTT, the area under the glucose curve progressively increased following 10, 20 and 30 days on HSD (935 ± 105; 1393 ± 144; 1496 ± 160 mM) whereas insulin secretion progressively decreased (1392 ± 127; 226 ± 78; 87 ± 34 pM). Histological evaluation of the pancreas following 10, 20 and 30 days on HSD revealed a gradual increase in exocrine fatty infiltration (2.5; 10; 15%), apoptotic index (1.9 ± 0.1; 4.8 ± 0.2; 5.0 ± 0.1% per total number of nuclei) and macrophages infiltration (0; scattered; massive), whereas pancreas weight gradually decreased (0.26 ± 0.03; 0.20 ± 0.02; 0.11 ± 0.02g). Islet morphology and insulin content were preserved. The isolated pancreas of CDs-HSD did not secrete insulin in response to glucose stimulation and both the first and second phase were undetectable. Following IVGTT of CDs fed HSD-Cu, the area under the glucose curve, insulin secretion, and pancreatic weight were 678 ± 80 mM; 905 ± 115 pM; 0.27 ± 0.01g, respectively. These values were similar to those of CDs fed 10 days HSD.
Conclusions: Our data suggests that exocrine dysmorphogenesis and β-cell dysfunction are progressive processes accompanied by fat and macrophages infiltration. Furthermore, we showed that these deleterious processes may be prevented by the supplementation of the HSD with adequate levels of copper. We conclude that impaired insulin secretion in CDs-HSD is not secondary to insulin resistance but rather related to the pathology of the exocrine pancreas, suggesting that exocrine endocrine interrelationships are important for glucose-responsiveness of the β-cells.
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Aspects of hepatic and peripheral insulin action

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Evidence for an indirect transcriptional regulation of glucose-6-phosphatase gene expression by liver X receptors

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Background and Aims: Liver X receptor paralogues alpha and beta (LXRα and LXRβ) are nuclear oxysterol receptors involved in the regulation of a number of genes responsible for cholesterol homeostasis. Recently the synthetic LXR-Agonist T0901317 (T09) has been shown to reduce the blood glucose level and improve insulin sensitivity in diabetic rodents. The decrease of blood glucose concentration was linked to transcriptional repression of key gluconeogenic enzymes like phosphoenolpyruvate carboxykinase (PEPCK) or glucose-6-phosphatase (G6Pase).
Our aim was to identify the mechanism by which liver X receptors repress the expression of the G6Pase gene.
Materials and Methods: G6Pase promoter activity and endogenous gene expression were analyzed in H4IIE rat hepatoma cells by G6Pase promoter reporter gene assays and Northern Blot experiments, respectively. LXRα and LXRβ were overexpressed using recombinant adenoviruses. Electrophoretic mobility shift assays (EMSA) were used to elucidate whether the G6Pase promoter contains LXR binding elements. Adenoviruses carrying siRNA with target sequences of SREBP1c or SHP will be used to further characterize the LXR effect.
Results: The LXR agonist T0901317 (T09) reduced both the endogenous level of G6Pase mRNA and the promoter activity in H4IIE rat hepatoma cells at a concentration of 3 µM by approximately 50%. Adenoviral mediated overexpression of LXRα or LXRβ demonstrated that each paralogue alone is sufficient to repress the endogenous G6Pase gene.
We demonstrated that the promoter fragment -151/+57 is able to confer the T09 effect on the gene promoter. This fragment and the entire G6Pase gene promoter were analyzed *in silico* for potential LXR binding elements. Five regions were identified but none of them bound overexpressed LXR in an electrophoretic mobility shift assay (EMSA). This indicates that LXR does not directly bind to the G6Pase gene promoter via a conserved LXR element. Preincubation of H4IIE cells with the translation inhibitor cycloheximide abolished the T09 effect which strengthens the hypothesis that the LXR effect is mediated indirectly. Further experiments revealed an induction of the transcription factor SREBP1c and the orphan nuclear receptor SHP by T09. Both proteins are candidates for G6Pase gene regulation. Currently we are investigating whether both proteins are necessary for LXR repression of G6Pase using adenoviruses with siRNA targeting SREBP1c or SHP.
Conclusion: The data reveal that adenoviral overexpression of LXRα or LXRβ is sufficient to reduce G6Pase gene expression. Furthermore we show that the G6Pase gene regulation by the LXR agonist T0901317 requires translation and we provide evidence for an indirect mechanism of action. Uncovering the exact mechanism of LXR mediated inhibition at the level of the G6Pase gene promoter could identify specific drug targets for the treatment of diabetes.

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FOXO1 as a master regulator of PPARγ and GLUT4 gene promoters in adipocytes: a novel paradigm to increase insulin sensitivity

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Background and Aims: Forkhead box O1 (FOXO1) and peroxisome proliferator-activated receptor gamma (PPARγ) are important transcription factors regulating glucose metabolism and insulin responsiveness in insulin target tissues. Reduced PPARγ levels and the receptor detachment from GLUT4 promoter upon thiazolidinediones binding, upregulate GLUT4 gene expression and increases insulin sensitivity, in bona fide insulin target cells. Therefore, we studied the effects of FOXO1 on the PPARγ and the GLUT4 gene expression in primary rat adipocytes.
Materials and Methods: Transcriptional regulation at gene promoter level was studied using expression vectors for wild type and mutant FOXO1, and luciferase-conjugated promoter reporters for PPARγ2, and GLUT4.
Results: Using RT-PCR, FOXO1, GLUT4, and PPARγ2 were all endogenously expressed in PRA but not in preadipose-like cells (CHOK1). FOXO1 coexpression in CHOK1 cells activated PPARγ2 promoter activity by 25-fold while repressing it by 60% in adipocytes. Further, the GLUT4 promoter was 2.5-fold activated by FOXO1 in PRA. Insulin and serum, two known upstream regulators of FOXO1, reversed FOXO1-mediated transcription in a tissue- specific manner. FOXO1 mutants defective in either the ligand-binding domain (H215R) or PKB/Akt-phosphorylation sites (T24A, S256A, S319A, AAA) partially or completely lost their effects on PPARγ2 and GLUT4 promoters. Progressive 5`deletion and gel retardation analyses revealed *cis*-elements on GLUT4 and PPARγ2 promoters, that mediate their regulation via specific binding of the FOXO1 protein.
Conclusion: We thus suggest a novel tissue-specific, insulin sensitive paradigm for FOXO1 regulation of PPARγ and GLUT4 gene promoters. In the preadipocyte state, increasing PPARγ level favours adipocytes maturation; in matured adipocytes the reduction of PPARγ level by FOXO1 on one hand, and FOXO1 activation of GLUT4 promoter on the other, enhance GLUT4 expression and thus insulin sensitivity. As unique FOXO1 response elements on GLUT4 and PPARγ2 promoters were identified, that mediate either repression or activation depending on the specific cell type, these sites may serve as a therapeutic target for type 2 diabetes.
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Orphan nuclear receptor small heterodimer partner represses hepatocyte nuclear factor 3/Foxa transactivation via inhibition of its DNA binding

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Small Heterodimer Partner (SHP; NR0B2) is an atypical orphan nuclear receptor and acts as a coregulator of various nuclear receptors. Herein, we examined a novel crosstalk between SHP and a forkhead transcription factor HNF3/Foxa. Transient transfection assay demonstrated that SHP inhibited the transcriptional activity of all three isoforms of HNF3, HNF3α, β and γ. In addition, SHP significantly repressed both basal and HNF3-mediated transactivation of *Glucose-6-Phosphase* (*G6Pase*) and *cholesterol 7-alpha-hydroxylase*(*CYP7A1*) promoters. Moreover, adenovirus-mediated overexpression of SHP decreased the mRNA levels of *G6Pase* and *CYP7A1*. *In vivo* and *in vitro* protein interaction studies showed that SHP physically interacted with HNF3. Confocal microscopic results demonstrated that SHP colocalized with HNF3 in the nucleus. In addition, HNF3 transactivation was still repressed by a SHP mutant which lacks the loop region between helices H6 and H7 required for both EID-1 recruitment and repression of nuclear receptors. Mapping of interaction domain revealed that SHP interacted with forkhead DNA binding domain of HNF3α. Gel mobility shift and chromatin immunoprecipitation assays demonstrated that SHP inhibits DNA binding of HNF3.
These results suggest that SHP is involved in the regulation of *G6Pase* and *CYP7A1* gene expression via novel mechanism of inhibition of HNF3 activity and expand the role of SHP as a coregulator of other family of transcription factors in addition to nuclear receptors.
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Effect of glutathion depletion on oxidative stress and insulin sensitivity in an experimental model of metabolic syndrome

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Background and Aims: Oxidative stress is associated with diabetes, cardiovascular disease and aging, but its role in the development of insulin resistance remains to be elucidated. Previously we have shown that nonobese hereditary hypertriglyceridemic rats with genetically fixed insulin resistance exhibited increased concentrations of lipoperoxidation products in serum and arterial wall and decreased levels of antioxidant enzymes. In this study we analyzed the potential of oxidative stress to induce insulin resistance in old hereditary hypertriglyceridemic rats (HHTg), a nonobese model of insulin resistance syndrome.

Materials and Methods: One year old male HHTg rats (body weight 410 ± 18 g) were subjected to oxidative stress by glutathione (GSH) depletion by means of the glutathione synthase inhibitor buthionine sulfoximine (BSO, 30 mmol/l in drinking water) for one week. The control group comprised age-matched HHTg (body weight 415 ± 21 g) rats with drug-free drinking water. All animals were fed a high sucrose diet (70 cal% as sucrose) diet for two weeks before the end of the study. Tissue sensitivity to insulin action was measured in vitro by incubation of tissues without or with insulin (250 µU/ml) according to basal and insulin-stimulated ^{14}C-U-glucose incorporation into soleus muscle glycogen or adipose tissue lipids.
Results: BSO treatment resulted in decreased plasma (3.74 ± 024 vs 10.69 ± 0.44 µmmol/l, $p < 0.01$, liver (5.29 ± 0.46 vs 11.48 ± 0.26 mmol/mg protein, $p < 0.01$) and myocardium (4.40 ± 14.39 mmol/mg protein, $p < 0.01$) GSH concentrations. The serum conjugated diene (46.7 ± 1.2 vs 30.5 ± 0.6 nmol/l, $p < 0.05$) and TBARS (4.30 ± 0.22 vs 3.24 ± 0.014, $p < 0.05$) levels were increased, indicating increased oxidative stress. BSO-treated animals exhibited also a reduction in serum triglyceride (1.8 ± 0.4 vs 4.2 ± 0.7 mmol/l, $p < 0.02$), ascorbic acid C (21.8 ± 2.0 vs 39.1 ± 1.99, $p < 0.05$) and α-tocopherol (8.8 ± 1.4 vs 11.3 ± 1.1, $p < 0.05$) concentrations. Fasting blood glucose (4.7 ± 0.1 vs 4.56 ± 0.2 mmol/l, N.S.) and serum insulin levels (536 ± 140 vs 455 ± 144 pmol/l, N.S.) in BSO rats were similar in BSO-treated rats to those of control rats.
On the other hand, GSH depletion impaired the tolerance to the oral glucose load (glycemia in 60 min.: 8.1 ± 0.4 vs 6.8 ± 0.1 mmol/l, $p < 0.05$; glycemia in 120 min.: 7.7 ± 1.6 vs 6.1 ± 0.18 mmol/l, $p < 0.05$; AUC_{0-120}: 918 ± 24 vs 778 ± 119 mmol/l/120 min., $p < 0.02$). Adipose tissue responsiveness to insulin was assessed in vitro by measuring 14C-U-glucose incorporation into lipids. BSO treatment was associated with adipose tissue resistance to insulin action measured in vitro according to ^{14}C-U-glucose incorporation into lipids during incubation without insulin (22.8 ± 1.5 vs 36.6 nmol//mg protein/2 hr, $p < 0.05$) or with insulin (32.2 ± 1.8 vs 56.8 ± 7.8 nmol//mg protein/2 hr, $p < 0.05$). In skeletal muscle low insulin responsiveness in old HHTg rats were not influenced by GSH depletion.
Conclusion: Results indicate that GSH depletion by BSO in old hereditary hypertriglyceridemic rats was associated with increasing of oxidative stress, impaired glucose tolerance and insulin resistance of adipose tissue. Our data support the hypothesis that increased oxidative stress can potentiate metabolic abnormalities associated with insulin resistance.
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Insulin resistance in adipocytes from ω3-fatty acid-depleted rats
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Background and aims: An insufficient supply of long-chain polyunsaturated ω3-fatty acids may favour insulin resistance. In such a perspective, the major aim of the present investigation was to characterize the regulation of adipocyte lipolysis and lipogenesis in ω3-fatty acid-depleted rats (2nd generation).
Methods: *In vivo* experiments were conducted in control and ω3-depleted fed male rats injected intravenously 60 min before blood sampling with 1.0 ml of either saline or a medium-chain triglyceride-rich emulsion. *In vitro* experiments were conducted in adipocytes isolated from control and ω3-depleted fed female rats and incubated for 60 min in the presence of D-glucose (8.3 mM) and, when so required, either theophylline (1.4 mM) or insulin (10 nM).
Results: When compared to control rats, the ω3-depleted rats displayed higher body and parametrial fat weight, normal glycemia and lipacidemia and a modest increase in insulinemia. As judged from the concentration and pattern of plasma unesterified fatty acids in rats injected intravenously with either saline or a lipid emulsion, the clearance of fatty acids from circulation was also higher in the ω3-depleted rats, whereas the inhibition of intracellular lipolysis caused by the injection of the lipid emulsion was less pronounced in the same rats. Likewise, in isolated adipocytes, both the uptake of [U-^{14}C]palmitate and the increase in lipolysis provoked by a phosphodiesterase inhibitor (theophylline), even when expressed relative to basal value, were higher in the ω3-depleted rats. Last, the rate of lipogenesis, as judged from the uptake of either D-[U-^{14}C]glucose or [1,2-^{14}C]acetate was lower in the adipocytes of ω3-depleted rats and, at variance with the situation found in control rats, not increased by insulin.
Conclusion: These findings reveal that ω3-depleted rats display obesity, increased clearance of circulating unesterified fatty acids and uptake of [U-^{14}C]palmitate by adipocytes, accelerated lipolysis whether *in vivo* or *in vitro* and, in terms of adipocyte lipogenesis, both a decrease in basal value and resistance to insulin, this coinciding with a modest increase in insulinemia. In all these respects, the situation found in ω3-depleted rats is comparable to that found in obese subjects with type 2 diabetes.

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Cytokine regulation of skeletal muscle metabolism: effect of interleukin-6 on palmitate oxidation rate and insulin sensitivity in the perfused hindlimb of the rat
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Background and Aims: Interleukin-6 (IL-6) has been regarded as a pro-inflammatory cytokine, but recent studies have shown that IL-6 may directly influence metabolic processes in skeletal muscles involved in the development of insulin resistance and type-2 diabetes. IL-6 has been shown to increase the rate of lipid oxidation in skeletal muscle *in vivo* and *in vitro* but whether IL-6 also affects insulin action in skeletal muscles is not clear.The aim of our study was to asses whether the above mentioned effect of IL-6 is present in an *in situ* model (the isolated and perfused rat hindlimb) where no neuro-endocrine response would interfere with IL-6.
Materials and Methods: All experiments were conducted using male Wistar rats (200–250 gram). The animals were surgically prepared for hindlimb perfusion and a Krebs-Ringer with 4% bovine serum albumin was used as perfusion buffer. Two different protocols were used to determine: 1) insulin sensitivity and 2) rate of palmitate oxidation. Insulin sensitivity was determined using a double isotope technique with 2-deoxy-^{3}H-Glucose and ^{14}C-mannitol, 8 mM glucose, 75 mU Insulin with or without 2500 ng/L IL-6. The hindlimb was perfused for 60 min ± IL-6. Immediately after the end of the perfusion biopsies of the soleus (SOL) red (RG) and white (WG) gastronemius muscles were taken out and frozen in liquid nitrogen for later estimation of glucose uptake. Palmitate oxidation rate was determined using ^{3}H-palmitate, 1 mM Palmitate, and 5 mM glucose with or without 5000 ng/L IL-6. Positive controls were preformed using 2 mM of 5-amino-4-imidazolecarboxamide riboside (AICAR), which previously has been shown to acutely increase the rate of palmitate oxidation. Several buffer samples were collected during the 120 min perfusion to determine the rate of palmitate oxidation.
Results: 2500 ng/L IL-6 (n=8) did not affect insulin stimulated glucose uptake compared to 0 ng/L IL-6 (n=7) in any of the muscles examined; SOL (9.5 ± 1.7 vs. 8.3 ± 2.0 µmol/g/hour, p=0.66), RG (6.7 ± 1.1 vs. 12.6 ± 3.9 µmol/g/hour, p=0.14), WG (3.7 ± 0.6 vs. 3.4 ± 0.6 µmol/g/hour, p=0.74). Furthermore 2500 ng/L IL-6 (n=6) did not affect the rate of palmitate oxidation compared to 0 ng/L IL-6 (n=5) (0.97 ± 0.06 vs. 0.86 ± 0.05 nmol/g/min). 2 mM AICAR (n=4) significantly increased the rate of palmitate oxidation compared to 0 mM AICAR (2.3 ± 0.08 vs. 0.86 ± 0.05 nmol/g/min, $p < 0.05$). All data are expressed as mean±SEM.
Conclusion: Under the present experimental conditions no acute effect of IL-6 on neither insulin sensitivity nor the rate of palmitate oxidation were found. This contradicts *in vivo* and *in vitro* studies which have demonstrated an increased rate of palmitate oxidation when IL-6 was administrated. In *in vivo* studies IL-6 significantly increased the plasma concentration of stress hormones such as cortisol making the interpretation of a direct effect of IL-6 more difficult. Results from *in vitro* studies were obtained using high supra physiological doses of IL-6. In conclusion our data suggest that some of the metabolic effects of IL-6 might be mediated through the neuro-endocrine pathway rather than through a direct effect on skeletal muscles or vasculature.
Support: Institute of Clinical Medicine, University of Aarhus;
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PS 40
Modulation of insulin action

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Glucosamine induces acute insulin resistance in muscle in vivo associated with impaired capillary recruitment

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Background and Aims: Glucose toxicity and glucosamine-induced insulin resistance have been attributed to products of glucosamine metabolism and direct effects of glucosamine on muscle metabolism have been demonstrated with incubated muscles. However, it is also possible that there is a haemodynamic element of glucosamine action with resultant impairment of both insulin-mediated limb blood flow and capillary recruitment, where inhibition of the latter has been shown to decrease insulin-mediated glucose uptake. In addition, endothelial cell nitric oxide synthase is inhibited by glucosamine. Since insulin has endothelial NO-dependent vasodilatory effects in muscle including capillary recruitment that enhance access for itself and glucose, the aim of this study was to investigate whether glucosamine-induced insulin resistance in muscle *in vivo* also involves an impairment in insulin's hemodynamic actions, particularly capillary recruitment.
Materials and Methods: *In vivo* experiments were carried out in anaesthetised rats following a 1 h equilibration after placement of cannulae. Rats were then infused for 3 h with glucosamine ($6.48\,mg \cdot kg^{-1} \cdot min^{-1}$), insulin ($10\,mU \cdot kg^{-1} \cdot min^{-1}$) or both. In rats receiving insulin, blood glucose was maintained at the initial level by the infusion of 30% glucose. At the end of the experiment blood was sampled from the femoral vein and carotid artery. From the arterio-venous difference multiplied by the flow, hind leg glucose uptake was calculated. A primed (2 µCi) continuous infusion ($0.1\,\mu Ci \cdot min^{-1}$) of [3-^{3}H]glucose was administered during the final 2 h. Arterial plasma samples taken at 165 and 180 min were de-proteinated, evaporated to dryness to remove ^3H_2O, resuspended and [^{3}H]glucose radioactivity determined. The rate of appearance (R_a) and rate of disappearance (R_d) of glucose were calculated. Femoral blood flow was continuously measured from a Transonic® flow probe. Capillary recruitment was determined by measuring the metabolism of infused 1-MX, a substrate targeted for xanthine oxidase.
Results: Glucosamine infusion alone increased blood glucosamine (1.9 ± 0.1 mM) and glucose (5.4 ± 0.2 to 7.7 ± 0.3 mM) ($P < 0.05$) but not insulin. Glucosamine induced both hepatic and muscle insulin resistance as evident from measures of R_a and R_d as well as hind leg glucose uptake which was inhibited by approx. 50% ($P < 0.05$). Insulin-mediated increases in femoral blood flow and capillary recruitment were completely blocked by glucosamine.
Conclusion: In summary, acutely administered glucosamine *in vivo* induced a state of muscle insulin resistance that was associated with markedly impaired haemodynamics where, in the presence of glucosamine, insulin did not significantly increase total flow or capillary recruitment. We propose that glucosamine inhibits insulin signaling in vascular cells and/or impairs endothelial NO production, thereby blocking the increases in total flow and capillary recruitment. In so doing impaired access for insulin and glucose may ensue to contribute to the insulin resistance similar to previous experimental situations where capillary recruitment by insulin has been blocked.
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No inhibitory effect of TNFα on insulin-stimulated glucose uptake in *in situ* skeletal muscle

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Background and Aims: Elevated plasma levels of the pro-inflammatory cytokine TNFα is strongly correlated to states of insulin resistance and obesity, however the exact role of TNFα is not fully understood. *In vitro* studies have shown that incubation of skeletal muscle tissue with TNFα does not impair insulin action. In contrast, recent *in vivo* studies in rats and humans, have shown that acute infusion of TNFα causes loss of insulin-stimulated capillary recruitment resulting in an attenuated muscular blood flow and diminished glucose uptake in skeletal muscle. It still remains to be established whether these reported *in vivo* effects of TNFα on vascular tissue and thereby impaired insulin action are due to a direct influence on the peripheral tissues or merely reflects a whole body pro-inflammatory neuro-endocrine response to the cytokine.
Since reduced insulin-stimulated glucose uptake in skeletal muscle is one of the most important features of the insulin resistant state, it is important to establish the exact role of TNFα in this particular tissue. Therefore, the present study was undertaken to investigate the effect of TNFα using the isolated and perfused rat hindlimb, where possible interactions with neuro-endocrine pathways are avoided.
Materials and Methods: The hindlimbs of male wistar-rats (200–250 g) were mounted and perfused with a heated (37 °C) and oxygenized (PaO_2=775–825 mmHg) Krebs-Ringer solution with 4% bovine serum albumin. Flow was has held constant at 0.083 mL min^{-1} g^{-1}. Perfusion-pressure, flow and PvO_2 were monitored continuously and glucose uptake was measured using a double isotope-technique.
Perfusion protocol: 20 min recovery period, 20 min perfusion with TNFα, 20 min perfusion with TNFα and insulin and finally 20 min perfusion with TNFα, insulin and isotopes (75 µCi/L 2-deoxy-^{3}H-glucose and 50 µCi/L ^{14}C -mannitol). TNFα was added in concentrations of 0, 250 or 2500 ng/L and insulin concentration was either 0, 75 or 500 mU/L. Muscle tissues mainly composed of type 1 (Soleus(Sol)), type 2a (Red Gastrocnemius (RG)) or type 2b (White Gastrocnemius (WG)) muscle fibers were studied.
Results: TNFα-administration had no effect on basal glucose uptake or insulin-stimulated glucose-uptake at either low or high insulin-concentrations in any of the examined muscle tissues. In addition, no effects on endothelial function or the mean vascular resistance during perfusion were seen (data not shown).

Table 1: Glucose uptake in the perfused rat hindlimb

TNFα-concentration	0 ng/L	0 ng/L	0 ng/L	250 ng/L	250 ng/L	250 ng/L	2500 ng/L	2500 ng/L	2500 ng/L
Muscle type	WG	RG	Sol	WG	RG	Sol	WG	RG	Sol
Glucose uptake, 0 mU/L insulin (µmol/hour/g muscle)	1.3 ± 0.3	2.6 ± 1.0	2.7 ± 1.1	no data	no data	no data	1.3 ± 0.5 (p=0.99)	1.5 ± 0.3 (p=0.24)	1.5 ± 0.4 (p=0.34)
Glucose uptake, 75 mU/L insulin (µmol/hour/g muscle)	3.4 ± 0.6	12.6 ± 3.4	8.3 ± 2.0	2.8 ± 0.5 (p=0.48)	11.0 ± 3.0 (p=0.75)	7.4 ± 2.4 (p=0.78)	3.2 ± 0.9 (p=0.80)	8.2 ± 3.0 (p=0.38)	8.3 ± 1.5 (p=0.98)
Glucose uptake, 30.6 ± 2.9 500 mU/L insulin (µmol/hour/g muscle)	17.8 ± 5.2	35.3 ± 4.9	26.7 ± 2.8	16.2 ± 3.9 (p=0.81)	42.5 ± 5.0 (p=0.33)	32.4 ± 3.9 (p=0.28)	18.3 ± 1.7 (p=0.91)	39.3 ± 2.6 (p=0.46)	(p=0.35)

Data are expressed as mean±SEM; n=7–8 in each group. All P-values are estimated with 0 ng/L as reference.

Conclusion: Our data show that in *in situ* skeletal muscle TNFα does not directly affect insulin-stimulated glucose uptake at either low or high insulin concentrations. The present data suggest that previously reported effects of TNFα on insulin-stimulated capillary recruitment and glucose uptake in skeletal muscle might require the presence of an intact neuro-endocrine system.
Support: Institute of Clinical Medicine, University of Aarhus; the Danish Medical Research Council

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Insulin sensitisation is triggered by a mixed meal but not by glucose – a study in conscious unrestrained animals

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Background and Aims: After a meal, activation of a pathway dependent on hepatic parasympathetic nerves (HPN) and NO enhances insulin action in the skeletal muscle. This process is absent in the fasted state. We tested the hypothesis that glucose is not the main trigger to the HPN-dependent mechanism and that two different kinds of meal result in different insulin sensitisation.

Materials and Methods: Male Sprague-Dawley rats, anesthetized using isofluorane (1-5%, inspired) and injected with ketoprofen (2.5 mg/kg, subcutaneous), received a gastric cannula and an arterial-venous (A-V) shunt (carotid artery-internal jugular vein). The A-V shunt allows intravenous (iv) drug infusions and arterial blood samples collection. The gastric cannula was used to administer food directly into the stomach (intragastric, ig). The catheters were tunnelled subcutaneously behind the shoulder blades, exteriorized and filled with heparin solution (200IU/ml). All surgical procedures were performed under sterilized conditions. After 6–10 days of recovery, the rats were fasted for 24 hrs prior to the experiment. Animals were conscious during the insulin testing. Insulin sensitivity was measured in the fasted state using a modified euglycemic clamp, in which glucose was given as a variable infusion (iv) in order to maintain euglycemia, after administration of a bolus of insulin (50 mU/kg, iv). Afterwards, using the gastric cannula, 10 ml/kg of either D-glucose (0.173 mg/ml, ig) or a liquid mixed meal (carbohydrate content: 0.173g/ml, ig) was given and a second insulin sensitivity clamp was performed. Atropine (1 mg/kg, iv) was administered and a third clamp performed.
Results: In both groups, the meal state induced an increase in insulin sensitivity, although only in the mixed meal group was this difference statistically significant (fasted vs fed state: mixed meal group, 132.1 ± 9.6 vs 250.3 ± 13.7 mg glucose/kg bw, n=7, P<0.001; glucose group, 117.7 ± 8.4 vs 162.6 ± 18.7 mg glucose/kg bw, n=6). Insulin sensitivity potentiation was significantly higher in the rats fed with the liquid mixed meal (94.0 ± 13.6%, n=7) than in those fed with the D-glucose solution (38.7 ± 11.6%, n=6; P<0.01). Atropine eliminated the insulin sensitization, returning the insulin sensitivity to the levels seen in the fasted state (139.2 ± 6.9 mg glucose/kg mixed meal, 115.0 ± 12.6 mg/kg glucose meal, not different).
Conclusion: In the fasted state, insulin sensitivity is decreased, which seems to be due to an inactive hepatic parasympathetic nerve-dependent component of insulin action. According to our data, D-glucose is not an effective feeding signal to cause insulin sensitivity, since the liquid mixed meal, which also contains proteins and lipids, besides carbohydrates, induced a significantly higher insulin sensitivity potentiation than the glucose did. Atropine reversed this effect, which confirms a hepatic parasympathetic nerve-dependent mechanism in meal-induced insulin sensitisation.
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Atropine inhibits postprandial insulin sensitivity in a dose dependent manner in humans
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Background and Aims: Insulin action is associated with the release of the Hepatic Insulin Sensitizing Substance (HISS), which enhances peripheral glucose uptake. HISS release in response to insulin is enhanced after a meal and is blocked by interfering with the hepatic parasympathetic nerves. In the immediate postprandial state HISS release is maximal, decreasing with the duration of fasting, both in rats and humans. Studies in rats showed that atropine induces the same degree of insulin resistance as seen with hepatic parasympathetic surgical denervation, suggesting that atropine is effective in eliminating the hepatic parasympathetic component of peripheral insulin action. We tested the hypothesis that the existence of a HISS-dependent component of insulin action in humans is regulated by a cholinergic mechanism in a dose dependent manner.
Materials and Methods: Six healthy male subjects (29.7 ± 3.2 years old) with normal values of fasting glycemia, insulin, C-peptide, lactate, HDL, LDL, cholesterol and triglycerides, were submitted to a single-blinded study. Insulin sensitivity was assessed by a modified euglycemic clamp technique to determine the glucose disposal produced by a bolus of 50 mU/kg body weight insulin. The volunteers were fasted for 14 h and after this period of time fed a standardized meal 100 min prior to starting the euglycemic clamp. The volunteers underwent a euglycemic clamp in three different days; intravenous infusions of either 0.50 mg, 0.75 mg atropine (low therapeutic doses with minor side effects) or saline (control group) were administrated 50 min before starting the euglycemic clamp.
Results: Thirty minutes before starting the euglycemic clamp, both insulin and glucose levels were stable and there were no differences between the control group and the two atropine groups (0.50 and 0.75 mg atropine dose). In the control group (saline infusion) the glucose disposal was 627.4 ± 83.9 mg glucose/kg body weight (n=6). In the atropine group, the glucose disposal decreased to 395.1 ± 64.3 mg glucose/kg body weight (n=6, P<0.01), for a dose of 0.50 mg of atropine and decreased to 287.3 ± 78.6 mg glucose/kg body weight (n=3, P<0.02) for a dose of 0.75 mg of atropine. Insulin sensitivity inhibition induced by atropine 0.75 mg infusion was higher (56.0 ± 8.1%, n=3) than with atropine 0.50 mg infusion (35.3 ± 8.1%, n=6, P<0.02).
Conclusion: Our data support the hypothesis that intravenous atropine administration results in HISS-dependent decreased insulin sensitivity in an atropine dose dependent manner, due to blockade of the hepatic parasympathetic nerves.
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Flavanol-rich dark chocolate improves insulin sensitivity, decreases blood pressure and ameliorates endothelium-dependent vasorelaxation, in patients with essential hypertension
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Background and Aims: Flavanols from cocoa improves nitric oxide (NO) release by vascular endothelial cells in vitro. In turn, increased NO bioavailability could ameliorate flow-dependent insulin sensitivity and decrease blood pressure (BP) and vascular inflammation in vivo. To address this topic, we compared the effects of both flavanol-rich and flavanol-free cocoa on glucose and insulin responses to oral glucose tolerance tests (OGTTs), systolic (S) and diastolic (D) BP levels, endothelium-dependent vasorelaxation and circulating inflammatory markers in both essential hypertensives and control subjects.
Materials and Methods: After a cocoa-free run-in phase of 7 days, 20 never-treated essential hypertensive patients (10 M, 43.65 ± 7.8 y) were randomly assigned to receive either 100 g/day flavanol-rich chocolate bars containing 100 mg of flavanols or 90 g/day flavanol-poor chocolate bars containing no flavanols, for 15 days, in isocaloric manner. Successively, subjects entered a further cocoa-free wash-out phase of 7 days and then were crossed over to the other condition. OGTTs were performed at the end of each period to calculate the homeostasis model assessment of insulin resistance (HOMA-IR), the quantitative insulin sensitivity check index (QUICKI) and the insulin sensitivity index (ISI). Ambulatory blood pressure monitoring (ABPM), office blood pressure (BP) measurement, flow mediated dilation (FMD) and ematochemical checks were performed at the same study phases. An identical study protocol was used for control group evaluation.
Results: Flavanol-rich but not flavanol-poor chocolate ingestion significantly decreased HOMA-IR (p<0.0001) and increased QUICKI and ISI (p<0.0001). Compared to baseline values one-factor repeated measures ANOVA showed higher FMD percent change after flavanol-rich (8.9 ± 1.4%, p<0.0001) but not flavanol-poor chocolate (7.5 ± 1.3%, n.s.) ingestion. BP significantly decreased after 15 days of flavanol-rich (systolic BP: – 11.0 ± 6.3 mmHg, p<0.0001; diastolic BP: - 6.2 ± 4.2 mmHg, p<0.0001) but not flavanol-poor chocolate ingestion (systolic BP: – 0.5 ± 1.6 mmHg, n.s.; diastolic BP: - 0.3 ± 3.1 mmHg, n.s.). ABPM data confirmed a significant BP reduction after dark (24 h SBP: – 11.9 ± 7.7 mmHg, p<0.0001; 24 h DBP: – 8.5 ± 5.0 mmHg, p<0.0001) but not white chocolate ingestion (24 h SBP: – 0.9 ± 2.7 mmHg, n.s.; 24 h DBP: – 0.1 ± 2.5 mmHg, n.s.). Similar variations for all parameters were observed in healthy control subjects. In addition, in hypertensive patients total cholesterol significantly decreased after flavanol-rich (p=0.0003) but not flavanol-poor chocolate ingestion.
Conclusion: Our results demonstrated that dark (flavanol-rich) but not white (flavanol-free) chocolate bars improved insulin sensitivity, decreased BP levels and ameliorated endothelium-dependent vasorelaxation in essential hypertensive patients. Our study represents the first demonstration that dark chocolate ingestion may have favourable effects on insulin sensitivity and BP. This supports the hypothesis that cardiovascular benefits may derive from dark chocolate ingestion, at least when careful attention is devoted to avoid an increased total caloric intake.

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Reduced glucose turnover is the primary effect of antiretroviral therapy, resulting in compensatory fat catabolism and visceral fat deposition

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Background and Aims: Highly active antiretroviral therapy (HAART) is known to cause changes in glucose and fat metabolism; HAART-treated patients develop a syndrome apparently similar to the metabolic syndrome: lipodystrophy, insulin resistance and diabetes. But the metabolic effects of these agents in noninfected subjects remain to be clarified. The goal of our study was to examine the development of the changes in glucose and fat metabolism in a canine model treated with the HIV protease inhibitor- Indinavir (IDV) and to compare with insulin resistance from visceral adiposity secondary to dietary fat.
Materials and Methods: Six normal dogs were given IDV 800 mg/TID (adult therapeutic dosage) for 6 weeks and assessed at week 0 and week 6 for 1) fat distribution by MRI and 2) insulin sensitivity and glucose turnover by a euglycemic hyperinsulinemic clamp.
Results:. By week 6 of IDV, fasting glucose turnover was decreased by 40%. (Basal: 2.78 mg/kg/min, sixth week: 1.64 mg/kg/min $p<0.05$) and the suppression of glucose production by insulin seen during the clamp was accelerated (total suppression at basal: 140 min, sixth week: 40 min). Also, fasting glucose was significantly decreased by the drug (–7% ($p< 0.01$)). MRI showed a tendency for increased fat (visceral and subcutaneous- a total increase of 17%). Basal FFA were increased by 50% by week 6 (Basal: 0.43 mmol/l, sixth week: 0.64 mmol/l $p<0.05$). Insulin, c-peptide and insulin sensitivity did not change significantly.
Conclusion: Decreased glucose output and enhanced plasma FFA reveal that Indinavir enhances conversion from carbohydrate metabolism to fat metabolism due to drug therapy alone. The conversion may result in elevated FFA and insulin resistance, which may in turn be responsible for the storage of extra fat, particularly in the visceral fat depot as reported for HIV patients on HAART therapy. Thus, the primary effect of this drug may be suppression of glucose output, followed by metabolic compensation (fat metabolism) and insulin resistance.

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Diabetogenic effects of some atypical antipsychotics: rapid, whole-body insulin resistance following a single dose

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Background and Aims: Recent evidence indicates that some atypical antipsychotics are associated with an increased risk of diabetes**.** While these drugs are also known to cause significant increases in body weight, a known diabetic risk factor, results of some studies suggest that antipsychotic-induced abnormalities in glucose metabolism can occur independently of weight gain. To investigate whether these drugs can cause insulin resistance without affecting body weight, we examined the acute effects of 4 atypical antipsychotics on whole-body insulin action.
Materials and Methods: We tested effects of acute (single dose) drug treatment using a pancreatic/insulin clamp (insulin 3 mU/kg*min; somatostatin 3 µg/kg*min) in male Wistar rats. The glucose infusion rate (GIR) to maintain euglycemia was monitored following a 5 hr fast. Once steady state was achieved, animals were administered a single s.c. dose of vehicle or drug, and GIR was monitored for 120 min.
Results: Clozapine (1, 3.2, 10 mg/kg) and olanzapine (1, 3.2, 10 mg/kg) caused a striking, dose-dependent ($P<0.001$) reduction in GIR (53–68% at maximal doses) within 40 min post-dose, consistent with whole-body insulin resistance. These effects were coincident with dose-dependent behavioral responses in drug-treated animals. In sharp contrast, ziprasidone (3.2, 10 or 32 mg/kg) and risperidone (2 mg/kg), antipsychotics with minimal and intermediate diabetic liability, respectively, produced similar dose-dependent behavioral effects, but had no effect on GIR.
Conclusion: Some, but not all, atypical antipsychotic drugs significantly impair whole-body insulin action, prior to any effect on body weight. Tracer studies are in progress to examine the mechanisms/sites of action (e. g. liver, muscle) of antipsychotic-induced insulin resistance. These data are highly relevant to ongoing scientific and regulatory examination of the increased diabetic risk of certain antipsychotics in a patient population with a predisposition for diabetes and metabolic disease.

PS 41
Adiponectin

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Circulating adiponectin is insulin-sensitive *in vivo*

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Background and Aims: Hyperinsulinemia and hypoadiponectinemia are risk factors for cardiovascular disease. We hypothesized that insulin would modulate circulating adiponectin *in vivo* and that this effect would be diminished in insulin-resistant subjects.
Materials and Methods: The adiponectin response to acute hyperinsulinemia was studied in sixteen otherwise healthy subjects (7 insulin-sensitive and 9 insulin-resistant men) in the course of a modified frequently-sampled intravenous glucose tolerance test (FSIGT). Glucose (0.3 g/kg) and insulin (0.03 U/kg) were injected iv at 0 and 20 min, respectively, and blood samples were drawn at defined intervals for glucose, insulin and adiponectin measurements. Insulin sensitivity (S_I) was computed by Bergman's minimal model, and areas under the curve (AUC) or under the baseline (AUB) for the different analytes were calculated.
Results: Serum insulin exhibited a first peak after glucose injection and a second, higher peak after the exogenous insulin administration. Serum adiponectin fell significantly after the first insulin peak in insulin-sensitive (mean decrease: 10.8 ± 6.6%, $P=<0.05$) but not in insulin-resistant subjects (mean decrease: 6.5 ± 5.7%, ns), and fell after the second insulin peak in insulin-sensitive (mean decrease: 21.3 ± 5.9%, $P<0.05$) and, to a lesser extent ($P<0.05$ for the difference between groups), in insulin-resistant subjects (mean decrease: 10.0 ± 5.8%, $P<0.05$).
Significant negative correlations were observed between both AUC_{ins} and AUC_{ins}/AUC_{glu} ratio and AUB_{adipo} (an integrated measure of the decline in adiponectin concentrations; r= -0.56, $P<0.05$ and r= -0.69, $P<0.005$, respectively).
These results suggest that insulin, glucose and adiponectin show an orchestrated regulation in the course of acute hyperinsulinemia *in vivo*. Insulin-resistant subjects, exhibiting higher AUC_{ins} and higher AUC_{ins}/AUC_{glu} ratios, are less sensitive to the inhibitory effect of insulin on adiponectin concentrations, which may be envisioned as a compensatory mechanism for their reduced insulin sensitivity.
Conclusion: In summary, insulin suppresses circulating adiponectin *in vivo*, but insulin-resistant subjects are less sensitive to this inhibition.
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Induction of adiponectin in skeletal muscle of *ob/ob* mice

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Background and aims: Adiponectin (ApN) is an adipokine exhibiting insulin-sensitizing, fat-burning and anti-inflammatory properties on several tissues including muscle. ApN could also modulate oxidative stress. This adipokine is almost exclusively produced by adipose tissue under normal conditions. However, ApN can be induced in the skeletal muscle in response to inflammatory stimuli. The aim of the present work was to examine whether ApN could also be upregulated in muscle in response to metabolic and/or oxidative aggression both *in vivo*, in a murine model of type 2 diabetes, and *in vitro*.
Materials and Methods: Obese, diabetic and leptin-deficient *ob/ob* mice (O, 13 wk old) were used and compared to lean littermates (L). A group of *ob/ob* mice was also treated with the antioxidant probucol for three weeks (P). On several occasions, tail vein blood was collected from fed animals. At the end of the experiment, *tibialis anterior* muscles were collected for measurement of mRNA levels by RTQ-PCR and of ApN levels by immunohistochemistry.
In vitro experiments were performed on C2C12 cells differentiated in myotubes. At day 4 of differentiation, different agents were added to the medium for up to 48 h in agreement with the experimental protocols.

Results: ApN gene expression was paradoxically increased in *tibialis anterior* muscle of *ob/ob* mice while it was decreased in their adipose tissue, with a lowering of circulating ApN as a consequence. The abundance of ApN mRNA in the muscle was ~80-fold higher in O mice than in L littermates (*P <0.001*) and proved to be positively correlated with plasma TBARS (thiobarbituric acid reactive substances) levels, a marker of lipoperoxidation and systemic oxidative stress. Immunohistochemistry clearly confirmed increased ApN labelling within myocytes of O mice. This technique also showed strong immunoreactivity for two markers of oxidative stress (PRDX3 and PRDX5) in obese muscle. Finally, ectopic lipid storage was detected in cryocut sections of *tibialis anterior* muscle from O but not L mice, using Oil Red O coloration.
We subsequently attempted to identify the mechanisms underlying muscle ApN induction *in vitro*. We tested on cultured myotubes the effects of glucose/lipid overload, high insulin and ROS to mimic the *in vivo* diabetic and obese situation where glucotoxicity, lipotoxicity, hyperinsulinemia/insulin resistance and/or oxidative stress prevail. High glucose (25 mM) and insulin (100 nM) did not affect ApN mRNA levels in C2C12 myotubes. By contrast, Structolipid® (a mixture of triglycerides) and ROS (reactive oxygen species) producers (H_2O_2 or the lipoperoxidation product, 4-hydroxy-2-nonenal) were efficient inducers of ApN when added to the C2C12 culture medium (~2.5-, ~2.5- and ~7-fold respectively, *P <0.001* for each). Besides, a ~11-fold rise in TBARS concentrations was measured in the medium of C2C12 cells after a 48 h-culture with the triglyceride mixture (*P < 0.05*). ApN induction was reversed by using an antioxidant. Thus, ApN upregulation was abolished with N-acetylcysteine *in vitro* and with probucol treatment of obese mice *in vivo*.
Conclusion: Adiponectin is upregulated in muscles of *ob/ob* mice. This induction may result from lipotoxicity and related oxidative stress. This overexpression could be viewed as a local protective mechanism to counteract ectopic fat deposition and oxidative damage in obese and diabetic muscle.
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Glucose attenuates human adiponectin gene expression and production *in vitro*

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Background and aims: Adiponectin is an adipocyte-specific protein, which has been implicated in the pathogenesis of insulin resistance. Furthermore, it has been shown that high levels of glucose augment insulin resistance (glucose toxicity). The aim of this study was 1) to investigate whether glucose has direct effects adiponectin gene expression and production in human adipose tissue (AT) *in vitro*, and 2) to investigate the possible molecular mechanisms behind these effects.
Materials and methods: Human subcutaneous AT fragments were used. Adiponectin mRNA was determined by RT-PCR, adiponectin protein by RIA, and interleukin-6 (IL-6) protein by ELISA. Nuclear factor-κB (NF-κB) activity was measured by EMSA.
Results: Glucose decreased adiponectin mRNA levels dose-dependently with an 80% reduction at glucose concentrations of 35 mM compared to 1 mM ($p < 0.05$). The reduced level of adiponectin mRNA was significant after 48 hours of incubation (2.8 ± 1.4 vs. 5.7 ± 2.2, $p < 0.05$), and remained reduced also after 72 hours ($p < 0.05$). The release of adiponectin protein was also reduced by high glucose concentrations (35 mM) compared to low glucose concentrations (1 mM) (26.6 ± 2.4 vs 32.5 ± 2.9 ng/ml, $p < 0.01$). In order to determine possible molecular mechanisms behind the glucose-induced inhibition of adiponectin we investigated the NF-κB pathway. High concentrations of glucose (35 mM) stimulated NF- κB activity ($p < 0.05$). Furthermore, the cytokines IL-6 (50 ng/ml) was found to inhibit adiponectin gene expression ($p < 0.05$), and high glucose (35 mM) stimulated the release of IL-6 2-fold ($p < 0.05$) compared to 1 mM glucose.
Conclusion: Glucose attenuates adiponectin gene expression and production in human AT *in vitro*. This inhibitory effect of glucose might play a role for the reduced levels of adiponectin observed in relation to states of high glucose levels such as type 2 diabetes mellitus. Furthermore, high levels of glucose increase NF-κB activation and the release of IL-6 in AT suggesting that NF-κB activation might be a possible mechanism whereby high glucose levels attenuate adiponectin gene expression and production.

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Increased gene expression for 11β-HSD1 and selected adipokines in subcutaneous adipose tissue of subjects with several features of metabolic syndrome without prediabetes

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Background and Aims: Obesity is considered as pivotal component of the metabolic syndrome. Nevertheless, the molecular mechanisms involved in the obesity-related insulin resistance, and in particular the role of several products of the adipose tissue are not yet well understood. Thus, the aim of the study was 1) to compare the gene expression for 11β-HSD1, leptin, TNFα, adiponectin, angiotensinogene and lipoproteine lipase in adipose tissue of healthy and obese insulin resistant subjects, and 2) to assess the effect of euglycemic hyperinsulinemia on the aforementioned parameters.
Materials and Methods: All recruited subjects initially underwent a 75g oral glucose tolerance test, and those with IFG, IGT and DM2 were excluded. Thus, 17 Caucasian men volunteers (8 lean; BMI: 22 ± 0.8, age: 29+2 yrs and 9 obese, drug naive subjects; BMI: 33 ± 0.9, age: 32+2 yrs) were included into the study. The *in vivo* insulin action was measured by a euglycemic hyperinsulinemic (1 mU/kg/min) clamp to reach steady state hyperinsulinemia around 100 μU/ml. Adipose tissue biopsies were performed twice, once after an overnight fast and then 2 days later, in the 3rd hour of euglycemic hyperinsulinemia. The mRNA levels of enzymes and adipokines of interest were assessed using quantitative real-time RT-PCR technique with the TaqMan Gene Expression Assays (Applied Biosystem, USA) and the 18S rRNA as housekeeper gene. Blood lipids and glucose were measured using standard biochemistry assays. Plasma insulin levels were measured using an ELISA (DRG GmbH, Germany) system.
Results: Clinical characteristics of studied groups are shown in Table 1. 11β-HSD1 mRNA level in subcutaneous adipose tissue was higher by almost 2 fold in the obese (O) insulin resistant subjects in comparison with controls (C) [O: 5.9 ± 0.6; C: 2.8 ± 1.1 AU, $p<0.05$]. Similar increase was found for leptin [O: 31.2 ± 2.9; C: 10.0 ± 2.5; AU, $p<0.001$] and TNFα [O: 10.0 ± 1.6; C: 4.7 ± 1.3 AU, $p<0.05$] mRNA levels. Nonetheless, we did not find any changes in the mRNA levels for adiponectin, angiotensinogene and lipoprotein lipase. In obese subjects, euglycemic hyperinsulinemia led to a further elevation of the mRNAlevel for 11βHSD1 [9.6 ± 1.0 $p<0.05$] and leptin (64.1 ± 12.5; $p<0.05$). On the contrary, there was no effect of hyperinsulinemia in the healthy controls.
Conclusion: Our data show that drug naive obese subjects with several features of metabolic syndrome, though yet without prediabetes, have increased gene expression for several enzymes and cytokines in the subcutaneous adipose tissue, and euglycemic hyperinsulinemia leads to further amplification of the changes seen. The latter may indicate toward a possible perturbation of the metabolic situation in response to endogenous hyperinsulinemia in the obese state.

Table 1: Clinical characterisics of studied subjects.

	Fasting Glucose mmol/l	120 min Glucose mmol/l	Fasting Insulin μU/ml	Insulin sensitivity mg/l/mU/min/kg	TG mmol/l	HDL mmol/l	Systolic mmHg	Diastolic mmHg
Controls	4.8 ± 0.2	5.4 ± 0.4	15 ± 5	0.17 ± 0.01	0.8 ± 0.2	1.3 ± 0.1	117 ± 5	68 ± 4
Obese	5.1 ± 0.2	6.7 ± 0.3*	27 ± 10	0.06 ± 0.01*	1.9 ± 0.4*	0.9 ± 0.1*	131 ± 4*	71 ± 2

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Hormonal regulation of AdipoR1 and AdipoR2 gene expression in 3T3-L1 adipocytes and in human adipose tissue

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Background and Aims: Adiponectin is synthesized and secreted exclusively by adipose tissue, and is reported to improve insulin sensitivity and atherosclerosis. We have recently identified the two adiponectin receptors, AdipoR1 and AdipoR2, in human adipose tissue, and found that the expression of these receptors were reduced in obesity and increased in association with weight loss. In the present study we investigate the influence of various

compounds and hormones that affect insulin sensitivity in 3T3-L1 adipocytes and human adipose tissue.
Materials and Methods: Fully differentiated 3T3-L1 cells were incubated with tumor necrosis factor-α (TNF-α) (10 ng/ml), interleukin-6 (IL-6) (50 ng/ml), interleukin-8 (IL-8) (1.0 μg/ml), dexamethazone (50 nM) and growth hormone (GH) (100 ng/ml) for 6, 24 and 48 hours. Moreover, the effect of the glitazone, troglitazone (0.1–20.0 μg/ml), was investigated in human adipose tissue fragments in incubations for 24 hours. Gene expression of AdipoR1 and AdipoR2 was quantified by RT-PCR and normalized to β-actin mRNA levels.
Results: In 3T3-L1 adipocytes both AdipoR1 and AdipoR2 mRNA were significantly suppressed by 38 to 74% when treated with TNF-α for 6 to 48 hours ($P<0.01$). GH induced AdipoR2 up to 2.5-fold ($P<0.01$), but had no effect on AdipoR1 mRNA. Other hormones affecting insulin sensitivity such as dexamethazone, IL-6 and IL-8 did not influence AdipoR1 and AdipoR2 gene expression in 3T3-L1 adipocytes. Troglitazone had no effect on AdipoR1 nor AdipoR2 mRNA in human adipose tissue fragments. TNF-α reduced AdipoR2 significantly by about 50% ($P<0.05$), but had no effects on AdipoR1 mRNA in human adipose tissue. As for 3T3-L1 cells, IL-6 did not influence AdipoR1 or AdipoR2 gene expression in adipose tissue fragments. Contrary, dexamethazone reduced AdipoR1 mRNA significantly by about 30% ($P<0.05$).
Conclusion: TNF-α is well known to induce insulin resistance, and in the present study we found that TNF-α is able to suppress AdipoR1 and AdipoR2 expression in a time-dependent fashion in 3T3-cells and at least also suppress AdipoR2 in human adipose tissue. This might further worsen the negative metabolic effect of low levels of adiponectin seen in states of increased levels of TNF-α and dexamethazone such as under severe stress and severe inflammation. The PPAR-γ agonist, troglitazone, had no effect on expression of AdipoR, indicating that the antidiabetic effects of troglitazone might not involve effects on these receptors in adipose tissue.

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Abstract withdrawn

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MCP-1 is regulated by adiponectin and exerts a key function in the negative crosstalk between adipose tissue and skeletal muscle in humans
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Background and Aims: Adipose tissue secretes a variety of factors that influence insulin sensitivity in skeletal muscle. The adipokine adiponectin is the only know positive regulator of insulin sensitivity. Its plasma concentration is decreased in obese and diabetic patients.
We recently showed that conditioned medium (CM) from differentiated human adipocytes impairs insulin signaling and GLUT4 translocation in human skeletal muscle cells. However, adipocyte-conditioned medium generated in the presence of adiponectin did not induce insulin resistance. Therefore, in the present study we analyzed the composition of adipocyte-conditioned medium generated in the presence or absence of adiponectin. Selected cytokines were then tested on skeletal muscle cells.
Materials and Methods: CM was generated using cultures of differentiated adipocytes derived from healthy subjects and treated with 5 nM adiponectin. Primary human skeletal muscle cells were treated with CM generated in the presence or absence of adiponectin and insulin signaling and GLUT4 translocation were assessed. The composition of CM was analyzed using cytokine protein arrays. Furthermore, skeletal muscle cells were treated with MCP-1 and other cytokines to analyze insulin signaling and glucose uptake. The expression of chemokine receptors was assessed by RT-PCR.
Results: Differentiated human adipocytes were found to secrete various cytokines including IL-6, IL-8, MIP-1α/β, TIMP1/2, GRO-α and MCP-1. Supernatants generated in the presence of adiponectin lost their ability to induce insulin resistance in skeletal muscle cells and exhibited reduced levels of these cytokines by up to 50%. Similar reduction of cytokine release by adipocytes was observed with AICAR. Thus, AMPK seems to play a pivotal role in the effect of adiponectin on adipocyte secretion.
Impairment of insulin signaling similar to that observed with adipocyte-conditioned medium was obtained by treatment of skeletal muscle cells with MCP-1 at doses ranging from physiological (150 pg/ml) to 100x physiological concentration, whereas IL-6, IL-8, resistin and TNF-α were effective at very high concentrations only. MCP-1 also significantly impaired insulin-stimulated glucose uptake in the myocytes. Expression analysis of chemokine receptors in skeletal muscle cells revealed the presence of CXCR2 and CCR1/2/4/5/10.
Conclusion: In conclusion, our data show that adiponectin downregulates the secretion of various adipokines. This could represent a mechanism by which adiponectin prevents impaired insulin action in skeletal muscle cells treated with adipocyte-conditioned medium. MCP-1 is regulated by adiponectin and impairs insulin signaling and glucose uptake in skeletal muscle cells at concentrations found in the circulation. Other cytokines that are released by adipocytes, however, significantly impair insulin action only at concentrations 50–5000-times higher than the physiological level. Therefore, MCP-1 may represent a key molecule in the negative crosstalk between adipose tissue and skeletal muscle.

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Adiponectin is an important determinant of apoA-I catabolism in both insulin-resistant and normal subjects
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Background and Aims: Adiponectin, synthesized in the adipose tissue, appears to play an important role in glucose and lipid metabolism. A positive correlation between HDL-cholesterol and plasma adiponectin levels has been demonstrated. However, the role of adiponectin in HDL metabolism remains unclear. This prompted us to perform a study in order to analyze the association between plasma adiponectin and the metabolism of apoA-I, the main apolipoprotein of HDLs.
Materials and Methods: An *in vivo* kinetic study with stable isotopes (^{13}C-leucine) was performed in 22 subjects including 11 insulin-resistant patients (8 type 2 diabetic patients, 3 non-diabetic obese subjects) and 11 normal individuals. Plasma adiponectin was measured by ELISA.
Results: Plasma adiponectin was significantly lower in insulin-resistant patients than in normal subjects (6.09 ± 2.08 vs. 8.80 ± 3.40 μg/ml, $p<0.05$). In the whole studied population, plasma adiponectin was positively correlated with HDL cholesterol ($r= 0.44$, $p<0.05$) and negatively with age ($r= -0.49$, $p<0.05$), BMI ($r= -0.45$, $p<0.05$), triglycerides ($r=-0.43$, $p<0.05$) and HDL triglycerides/cholesterol ($r= -0.42$, $p<0.05$). ApoA-I Fractional Catabolic Rate (FCR) was significantly increased in insulin-resistant patients compared to normal subjects (0.24 ± 0.06 vs. 0.18 ± 0.025 pool/day, $p=0.015$). A strong negative correlation was found between plasma adiponectin and apoA-I FCR ($r=-0.66$, $p<0.001$) in the whole studied population as in the group of insulin-resistant patients ($r= -0.72$, $p=0.012$) and in the group of normal subjects ($r=-0.68$, $p=0.023$). In multivariate analysis, apoA-I FCR was significantly associated with plasma adiponectin ($p=0.0023$) and HDL triglycerides/cholesterol ($p=0.0025$), but not with age, sex, BMI and plasma triglycerides. Both plasma adiponectin and HDL triglycerides/cholesterol explained 66% of the variance of apoA-I FCR.
Conclusion: 1) Our kinetic study has shown a strong negative correlation between plasma adiponectin and apoA-I FCR in both insulin-resistant and normal subjects. This can explain the positive correlation between HDL-cholesterol and plasma adiponectin. 2) The association between adiponectin and apoA-I FCR is independent of the content of triglycerides within HDLs. 3) These data suggest a possible role of adiponectin in HDL catabolism.

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Effect of LMNA gene 1580G/A mutation on 3T3 F442A adipocytes differentiation and gene expression

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Background and Aims: *LMNA* gene encodes for lamin A/C, a nuclear envelope protein involved in nuclear structure and function. The homozygous 1580G/A missense mutation of *LMNA* gene (accession number X03444), which changes lamin A/C arginine 527 in histidine (R527H), is responsible for the mandibuloacral dysplasia (MIM 248370) an autosomal recessive complex disorder which includes partial lipodystrophy, impaired glucose tolerance and type 2 diabetes.
The aim of our study was to asses the effect of LMNA 1580G/A mutation in adipocyte differentiation and function.
Materials and Methods: To this purpose, 3T3 F442A preadipocytes have been stably infected with wild type (WT) and mutated (MUT) human lamin A/C cDNA inserted in viral vector pLNCX2; empty vector was used as a transfection effect control (ctrl), while wild type cells were used as a negative control (NC). Transfection efficiency was controlled by RT-PCR of human lamin A/C gene expression. Cell were grown to confluence in DMEM, 10% NCS, 2 mM glutamine, and differentiation was induced with DMEM, 10% FBS, 10^{-6} M Insulin. Cell differentiation was assessed by microscopic observation and Oil-Red O staining. Total RNA was extracted from cells at 0, 3, 5, 7, 10 days of differentiation, and real time PCR (Applied Biosystems) was performed to quantitate adipogenic transcription factor and adipocytokines gene expression in three different experiments.
Results: No significant differences were observed between NC, ctrl, WT and MUT transfected cells differentiation as assessed by Oil-Red O staining. Lamin A/C overexpression induced a general increase of gene expression during differentiation probably reflecting a putative role of this protein in transcription regulation.
In particular we observed an increase of 1.5 to 2 fold of PPARγ expression in WT transfected cells as compared to ctrl respectively at 7 and 10 days of differentiation ($p<0.05$), while MUT cells showed a lower and delayed increase in expression: 1.4 fold change at day 10 as compared to ctrl ($p<0.05$). Similarly, SREBF-1c and cEBP-α gene expression in WT and MUT cells showed a statistically significant increase only at day 10: 1.6 fold change ($p= 0.02$) for both conditions.
As for the adipokines expression, WT and MUT cells showed an inverse regulation of adiponectin and TNF-α: in fact adiponectin expression increment at day 7 and 10 was higher than in control cells (WT: 1.4 and 3.1; MUT 1.2 and 2.5 fold change respectively at day 7 and 10; $p<0.01$), whereas in MUT cells TNF-α, whose expression was higher than in ctrl and WT cells at day 3 (~2 fold change; $p<0.01$), was greatly decreased at day 7 (2 fold change; $p<0.05$).
In WT and MUT cells leptin expression was lower than in control cells in the first days of differentiation, whereas it showed a greater increment at days 7 and 10 (WT: 1.3 and 4.2; MUT 1.5 and 6.3 fold change respectively at day 7 and10; $p=0.02$).
Conclusion: In conclusion, our data show that lamin A/C overexpression in 3T3-F442A cells induces a delayed and incomplete differentiation as assessed by the shift in adipogenic transcription factors expression; this effect was enhanced in MUT cells. This delay is paralleled by higher TNF-α levels and lower leptin levels in the first days of differentiation, whereas adipokines levels tend to reach normal range later during the differentiation process.

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Fibroblast growth factor-1 (FGF-1) as a potential therapeutic target for obesity: mechanisms of FGF-1 action in human preadipocytes

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Background and Aims: Obesity is a disease of increasing prevalence with a wide range of negative health and social implications, however the mechanisms governing adipose tissue mass regulation are not fully characterised. We have previously identified that FGF-1 dramatically increases both the proliferation and differentiation of human primary preadipocytes (hPA) and that treatment with FGF-1 inhibitors significantly reduces these effects. FGF signalling pathways may therefore be potential targets for anti-obesity therapies. The objectives of this study were to further define the mechanisms of FGF-1 action in hPA and to identify the FGF signalling pathways involved in this process.
Materials and Methods: hPA were grown and differentiated in the presence or absence of FGF-1. The expression of adipogenic transcription factors and FGF receptors was determined at confluence and during differentiation by real time RT-PCR and western blotting. The levels of differentiation were assessed morphologically, by enzymatic assay of the differentiation marker G3PDH and by western blotting of perilipin and GLUT4. Functionality of the differentiated hPA was determined by measuring adiponectin and leptin secretion by RIA, and by assessing insulin's effects on 2-deoxyglucose uptake and isoproterenol induced lipolysis. The activation of common FGF signalling pathway molecules PLCγ1, p38 MAPK, p42/44 MAPK and Akt was investigated by western blotting with phospho-specific antibodies.
Results: Treatment of hPA with FGF-1 during proliferation had a priming effect on preadipocytes, resulting in upregulation of the adipogenic transcription factor PPARγ at confluence, prior to induction of differentiation. Following differentiation, up to 90% of FGF-1 treated preadipocytes accumulated lipid whereas only 5–20% of cells accumulated lipid in the absence of FGF-1. There was also a corresponding increase in the expression of PPARγ, C/EBPα, C/EBPβ and differentiation markers perilipin and GLUT4 during differentiation in the presence of FGF-1. Preadipocytes differentiated in the presence of FGF-1 secreted high levels of adiponectin and leptin and were insulin responsive, exhibiting a 3-fold increase in glucose uptake and protection from isoproterenol induced lipolysis. Human preadipocytes expressed FGF receptors 1, 2, 3 and 4. While FGFR2 and FGFR3 expression was increased in FGF-1 treated preadipocytes, FGFR1 and FGFR4 expression was unchanged. Of the signalling pathways examined, p42/44 MAPK was activated by FGF-1 during proliferation and in the early stages of differentiation, suggesting that the MAPK pathway may be involved at discrete stages.
Conclusion: FGF-1 increases commitment of human preadipocytes to the adipocyte lineage and upregulates the adipogenic transcription program, resulting in functionally active adipocytes. FGF-1 treated human preadipocytes are therefore a good model to study the features of both human preadipocyte differentiation and insulin signalling. Furthermore, these results demonstrate the importance of FGF-1 signalling in human preadipocyte differentiation and indicate that further characterisation of the mechanisms of FGF action in human adipogenesis may allow for the development of potential therapies for obesity.
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Increased expression and altered in vivo regulation by insulin characterise multiple monocyte/macrophage genes in insulin resistent human adipose tissue

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Background and Aims: Expression of macrophage markers is increased in adipose tissueof obese mice. We explored whether this is also true in insulin resistant human subjects and whether insulin acutely regulates macrophage marker expression in human adipose tissue in vivo.
Materials and Methods: 21 non-diabetic women were divided into insulin sensitive (IS) and insulin resistant (IR) groups based on their median whole body insulin sensitivity (8.7 ± 0.4 vs. 4.2 ± 0.3 mg/kg·min for IS vs. IR) measured using the euglycemic hyperinsulinemic clamp technique. The IR women were matched for age with the IS women but were more obese (BMI 24.7 ± 1.1 vs. 32.7 ± 1.8 kg/m^2, IS vs. IR, $p<0.01$). Subcutaneous adipose tissue biopsies were obtained before and after a 6 hr insulin infusion and adipose tissue mRNA concentrations of CD68 (macrophage specific marker), EMR1 (macrophage-restricted glycoprotein), ITGAM (integrin found in monocytes, macrophages, neutrophils and NK cells), ADAM8 (disintegrin-like metalloproteinase expressed in monocytic cells), CCL2 (monocyte chemoattractant protein, MCP-1) and CCL3 (macrophage inflammatory protein) were measured using real-time PCR and expressed relative to housekeeping gene RPLP0.
Results: At 0 hrs, whole body insulin sensitivity was significantly correlated with mRNA concentrations of CD68 (Spearman's $r=-0.56$, $p<0.01$), EMR1 ($r=-0.62$, $p<0.01$), ITGAM ($r=-0.50$, $p<0.05$), ADAM8 ($r=-0.42$, $p=0.058$)

and CCL3 (r= –0.62, p<0.01). In IS subjects, insulin significantly decreased the expressions of CD68 and ITGAM whereas there were no changes in these genes in IR subjects. In both IS and IR subjects, insulin increased expression of CCL2. At 6 hrs, all measured genes were significantly inversely correlated with whole body insulin sensitivity [CD68 (Spearman's r= –0.69, p<0.001), EMR1 (r= –0.56, p<0.01), ITGAM (r=-0.60, p<0.01), ADAM8 (r=-0.67, p<0.001), CCL2 (r= –0.60, p<0.01) and CCL3 (r= –0.73, p<0.001)].
Conclusion: Insulin resistance in vivo is associated with increased expression of multiple monocyte/macrophage genes in human adipose tissue. Insulin downregulates expression of macrophage specific genes in IS subjects but not in IR subjects. These data provide direct evidence of inflammation in human adipose tissue and extend insulin action to regulation of gene expression in macrophages.

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Elevation of free fatty acids induces TNF-alpha and IL-6 gene expression in human subcutaneous fat of obese subjects. Role of macrophage infiltration

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Background and Aims: In obesity, adipose tissue is chronically exposed to a positive energy balance, thus becoming hypertrophic and functionally impaired to further take up free fatty acids (FFA) in excess as well as to differentiate. Furthermore obesity is associated with a mild chronic inflammatory state and adipose tissue is the site of production of cytokines involved in the pathogenesis of obesity-associated insulin resistance.
Aim of our study was to assess the effect of FFA on gene expression of adipogenic transcription factors and adipokines and to evaluate the role of macrophage specific gene expression in the inflammatory drift of adipose tissue
Materials and Methods: To this purpose, a subcutaneous adipose tissue needle biopsy was performed before and after a 5-hr lipid infusion (Intralipid + heparin) in 8 obese non-diabetic women (aged 40.8 yr; range 25–59; BMI 39.0 ± 7.6).
After collagenase digestion of subcutaneous adipose tissue obtained from five different patients (2/3 M/F; aged 36.5 yr; range 28–53; BMI 43.2 ± 6.5), adipocyte fraction was collected and macrophages were extracted from stromal vascular fraction through antibody conjugated magnetic beads column separation.
Quantification of mRNA expression was carried out by Real Time PCR.
Results: Plasma FFA concentration rose from 311.5 ± 70.7 to 1600.0 ± 146.1 μM. Unexpectedly, mRNA expression of PPARγ2, C/EBPα and SREBP1c decreased after lipid infusion by 36.5%, 15% and 51.6% respectively; however, only SREBP1c decrement reached statistical significance (3.1 ± 0.25 vs 1.5 ± 0.30, mean±sem of relative amounts, p=0.019). As for the adipokines mRNA data, leptin showed a not significant decrement (36.4%), resistin rose by 25% (P=NS), while adiponectin remained unchanged. On the contrary, both IL-6 and TNFα gene expression markedly and significantly increased after lipid infusion: 1.9 ± 0.45 vs 3.6 ± 0.7, p=0.01 and 0.24 ± 0.06 vs 0.54 ± 0.12, p=0.005, respectively). Cytokines mRNA quantification from macrophages and adipocytes derived from the same adipose tissue sample, showed similar expression from both cell types. Therefore, in sc adipose tissue of obese women: 1) in vivo gene expression of adipogenic transcription factors is slightly downregulated by lipid infusion; 2) leptin, adiponectin and resistin are only mildly affected whereas, 3) IL-6 and TNFα are markedly upregulated by high FFA plasma levels. Adipocytes and adipose tissue macrophages are both sites of expression of IL-6 and TNFα.
Conclusion: In conclusion, these data show that high FFA in obesity induce overexpression of proinflammatory cytokines and suggest that it might contribute to the derangement of adipose tissue function and differentiation and to the chronic inflammatory condition associated with insulin-resistance.
Furthermore, although infiltrating macrophages contribute to cytokine production in adipose tissue, adipocytes per se show high level of expression of inflammatory cytokines.

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Expression of TWEAK and its receptor Fn14 in human subcutaneous adipose tissue. Relationship with other inflammatory cytokines in obesity

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Background and Aims: Recent studies from lean and obese animal models have pointed out that many inflammatory genes are upregulated in obesity. TWEAK, a cytokine of the TNF- family, has been found to be expressed under different inflammatory conditions but no data is available concerning the expression of this cytokine and its receptor (Fn14) in human obesity. Macrophage infiltration of adipose tissue is a recent described finding. In the present work we aim to evaluate the expression of two TNF system cytokines: TNF-like weak inducer of apoptosis (TWEAK) and tumor necrosis factor α (TNF-α) and their receptors, Fn14 and TNFR1, TNFR2 respectively, in human subcutaneous adipose and its relation with inflammation, the presence of type 2 diabetes, by also measuring the expression of macrophage marker CD68.
Materials and Methods: Gene expression levels of TWEAK, Fn14, TNF-α, TNFR1 and TNFR2 were measured from subcutaneous adipose tissue from eighty-four subjects with different degree of obesity by semiquantitative Real Time PCR. Serum levels of sTNFR1 sTNFR2 were also measured. Mature adipocytes and stromovascular fraction was separated from 3 morbidly obese tissue samples in order to check for TWEAK and Fn14 expression. The effect of inflammation on the expression of TWEAK and Fn14 was studied *in vitro* on THP-1 monocytic cell line.
Results: TWEAK was found to be expressed in adipose tissue of all studied subjects with no differences between obesity group, and was associated with Fn14 expression in morbid-obese, mainly in women with type 2 diabetes. We detected expression of TWEAK and Fn14 in isolated mature adipocytes and in the stromovascular fraction. Additionally, we found that LPS upregulates the expression of both genes on THP-1 human monocytic cell line.
TNF-α and TNFR2 mRNAs were also significantly more expressed in subcutaneous adipose tissue of subjects with morbid obesity compared to obese and non-obese subjects. In contrast, subjects with morbid obesity showed significantly lower TNFR1 gene expression. CD68 gene expression correlated positively with BMI.
Conclusion: Morbidly obese patients have a more severe pro-inflammatory milieu with a preponderance of macrophage marker. The presence of new pro-inflammatory cytokines like TWEAK in mature adipocyte cells reinforces the role of the adipocyte as a source of inflammatory molecules that may be activated in presence of macrophage infiltration.
This work has been supported by grants from: Marato TV3 (02/19190); Fondo de Investigación Sanitaria (FIS 02/3033 and FIS 04/0377), Red de Centros (C03/08) and Red de Grupos (G03/212)

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Depot-specific differences in adipocyte biology and adipose tissue gene expression in humans and mice

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Background and Aims: Visceral and subcutaneous adipose tissue display important metabolic differences that underlie the association of visceral obesity with insulin resistance and the risk of cardiovascular disease. In vitro, visceral adipose tissue exhibits increased lipolytic activity and decreased insulin sensitivity as compared to subcutaneous fat and this is associated with intrinsic differences in gene and protein expression.
Materials and Methods: Using Affymetrix microarrays, we compared gene expression between visceral and subcutaneous adipose tissue of C57Bl6 mice and in parallel adipose tissue obtained from different depots in humans.
Results: We found 134 genes in mice and 128 genes in humans, which were significantly differentially expressed in a fat depot-specific pattern. Of these genes, 72 genes were identified both in mice and in humans (25 genes higher in subcutaneous fat and 47 genes higher in visceral fat), including fatty acid synthase, lamin A, β-adrenergic receptors, PKC β and δ. Further analysis revealed a coordinated down-regulation at key steps in insulin sig-

naling, glucose transport (including lower GLUT-4 gene expression) and glycolysis in visceral adipocytes. Reflecting the increased lipolytic activity in visceral fat, there was a coordinated up-regulation of genes coding for lipolytic enzymes, including carnitine palmitoyl transferase 2. Moreover, expression of secreted molecules including leptin, IL-6, TGFβ, and angiotensinogen was significantly different between visceral and subcutaneous adipocytes, suggesting a distinct endocrine function of visceral adipose tissue.
Conclusion: Taken together, our data suggest that intrinsic differences in gene expression between visceral and subcutaneous adipose tissue are evolutionary conserved and could contribute significantly to a causal link between visceral obesity and increased cardiovascular and metabolic risk.
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Tetradecylthioacetic acid (TTA) and fish oil (FO) decrease the gene expression for lipoprotein lipase in adipose tissue of rats with high-fat diet induced insulin resistance
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Background and Aims: Our previous results have shown that TTA, a non-β-oxidizable fatty acid (FA) analog, and marine FO, rich in 3-n PUFAs, improve insulin sensitivity through increased FA oxidation in liver and muscle. Emerging knowledge on the importance of adipose tissue as an endocrine organ able to produce various adipokines influencing insulin sensitivity led us to evaluate the effect of the aforementioned hypolipidemic agents from this perspective. Therefore, the aim of our study was to investigate the effect of TTA and FO on gene expression for lipoprotein lipase (LPL) and adiponectin in epididymal adipose tissue of rats with high-fat diet-induced insulin resistance in 1) the basal state and 2) after euglycemic insulin stimulation.
Materials and Methods: Male Wistar rats were fed a basal (PD) or a 70 cal% high-fat diet (HF), or a HF diet supplemented with 10 wt% FO (HF+FO) or TTA (200 mg/kg/day) (HF+TTA). Expression of genes for LPL and adiponectin in epididymal adipose tissue was measured after a 16 hour fast or after 90-minute euglycemic hyperinsulinemia (EHC, insulin infusion rate 6,4 mU/kg/min) using the real-time RT-PCR (TaqMan gene expression assays, Applied Biosystems) technology. Expression levels presented in arbitrary units were normalized to 18S rRNA.
Results: Feeding the rats HF diet led to increased expression of LPL in epididymal adipose tissue (PD: 1.1 ± 0.1; HF: 2.3 ± 0.4; p=0.011). Supplementation with FO slightly decreased the level of LPL mRNA (HF+FO: 1.6 ± 0.1; p=NS) and treatment with TTA normalized the values to the control animals (HF+TTA: 1.3 ± 0.2; p=0.035). LPL expression correlated with adipose tissue mass (r=0.59, p=0.003) and with fasting plasma leptin concentration (r=0.71, p<0.001). Animals subjected to EHC showed higher levels of LPL mRNA when compared to the animals in basal state (PD: 5.7 ± 1.7; HF: 8.7 ± 2.3; HF+FO: 7.2 ± 1.9 and HF+TTA: 6.3 ± 1.3; p<0.05), however, no differences were observed among the individual treatment groups. Adiponectin expression in epididymal adipose tissue was not effected by any of the dietary treatments, and no correlation was found with any of the physiological parameters studied.
Conclusion: In summary, our data indicate that dietary supplementation with hypolipidemic agents like TTA and FO in high fat diet induced insulin resistance is associated with regulation of lipid metabolism in epididymal adipose tissue at the level of LPL, in particular by decreasing the expression of this lipolytic enzyme. Insulin stimulation of LPL expression is not influenced by TTA or FO treatment. Thus, the changes of LPL gene expression in adipose tissue might play a role in insulin-sensitizing effect of TTA and FO.
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Overexpression of Kruppel-like factor 7 regulates adipocytokine gene expressions in human adipocytes, and inhibits glucose-induced insulin secretion in pancreatic beta cell line
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Background and Aims: We have identified Kruppel-like factor 7 (KLF7) as a new candidate for conferring susceptibility to type 2 diabetes. To ascertain the possible involvement of KLF7 in the pathogenesis of type 2 diabetes, we examined the functional roles of KLF7 in human adipocytes and in the insulin secreting cell line.
Materials and Methods: The human adipocytes were infected with adenovirus vectors encoding human KLF7, and the expression of genes encoding adipocytokines was determined by real-time quantitative PCR. The secretion of adipocytokines into culture media was determined by enzyme-linked immunosorbent assays (ELISA). In the insulin secreting cell line (HIT-T15), mRNA and protein content of insulin were determined by real-time quantitative PCR and by ELISA, respectively. The effect of KLF7 on glucose-induced insulin secretion into culture media was also examined.
Results: In human adipocytes overexpressing KLF7, the mRNA expression and protein secretion of adiponectin and leptin were decreased compared to those in control cells, whereas expression and protein secretion of IL-6 were increased. In the insulin secreting cell line (HIT-T15 cells), the expression and glucose-induced secretion of insulin were significantly suppressed in KLF7 overexpressed cells compared to control cells, accompanied by the reduction in the expression of GLUT2, SUR1, Kir6.2 and PDX1.
Conclusion: These results suggest that KLF7 may contribute to the pathogenesis of type 2 diabetes through an impairment of insulin biosynthesis and secretion in pancreatic beta cells, and a reduction of insulin sensitivity in peripheral tissues. Therefore, we suggest that KLF7 plays an important role in the pathogenesis of type 2 diabetes, and may be a useful target for new drugs to aid in the prevention and treatment of this disease.

Effects of KLF7 on the expression of adipocytokine genes. * P<0.05, ** P<0.01 vs. LacZ

	Adiponectin	Leptin	IL-6
LacZ	1.072 ± 0.112	1.158 ± 0.125	1.217 ± 0.241
KLF-7	0.407 ± 0.059 **	0.241 ± 0.012 **	14.206 ± 5.219 *

Effect of KLF-7 on glucose-induced insulin secretion. * P<0.01 vs. LacZ

	0 mM	2.7 mM	10 mM	16.7 mM
LacZ	7.910 ± 1.060 (100)	10.095 ± 0.906 (138 ± 8)	11.946 ± 0.556 (151 ± 15)	14.990 ± 1.221 (190 ± 17)
KLF-7	5.141 ± 1.330* (100)	5.057 ± 0.643* (98 ± 12)	5.439 ± 1.313* (106 ± 2)	5.131 ± 0.609* (100 ± 15)

PS 43
Aspects of insulin therapeutics

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Insulin glulisine and human insulin have similar mitogenic potential *in vitro* and *in vivo*

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Background and aims: Structural changes in the amino acid sequence of human insulin (HI), as in analogues of HI, can be associated with changes in the receptor binding properties and their proliferative potential. In light of this, the mitogenic potential of insulin glulisine (GLU), a new rapid-acting insulin analogue, was compared to Asp(B10) and HI *in vitro* and *in vivo*.
Materials and methods: The induction of DNA synthesis was assessed by [^{3}H]-thymidine incorporation in the human epithelial breast cell line MCF10. The interaction with the insulin-like growth factor 1 (IGF-1) receptor, DNA synthesis and intracellular signal transduction were assessed in cardiac K6 myoblasts. A histopathological examination of all tissues and a retrospective immunohistochemical examination of the mammary glands from Sprague-Dawley rats treated with GLU for 6 months (n=40), and GLU and HI for 12 months (n=60), was also performed *in vivo* to identify any proliferative activity.
Results: GLU was less potent in stimulating DNA synthesis compared with HI in the human cell line MCF10, while Asp(B10) was slightly more potent than HI. Furthermore, GLU had a reduced binding affinity relative to HI in K6 myoblasts. GLU demonstrated higher IGF-1 receptor autophosphorylation compared with HI; however, similar levels of Shc protein activation were observed with GLU and HI, and phosphorylation of MAP kinases (ERK1 and ERK2), which has been related to mitogenic activity, was lower with GLU than with HI. Stimulation of DNA synthesis was comparable for GLU and HI in K6 myoblasts. At 12 months, there was no significant difference between the GLU and HI groups with respect to proliferative activity in rat mammary gland tissue, and the incidence of mammary tumors was not different in both groups.
Conclusions: We conclude that the mitogenic potential of GLU is similar to that of HI *in vitro*, whereas this is greater with Asp(B10). This was confirmed by the *in vivo* data, which showed that GLU and HI are not different in the proliferative effect on mammary glands, in contrast to findings reported with Asp(B10).
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Effects of insulin glulisine on the insulin and insulin-like growth factor 1 receptor signalling cascades

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Background and aims: Insulin glulisine (GLU) is a new, rapid-acting insulin analogue. The aim of the studies presented here was to compare the effects of GLU with human insulin (HI) and the insulin analogue Asp(B10) (which has been shown to have increased mitogenic activity via the insulin receptor), in terms of receptor binding and signalling in various *in vitro* assay systems.
Materials and methods: Insulin and insulin-like growth factor 1 (IGF-1) receptor binding was studied with HI receptors from 293HEK cells and the human osteosarcoma-derived cell line B10. Insulin receptor-mediated signalling was assessed in rat-1 fibroblasts over-expressing insulin receptors. Activation of the insulin receptor substrates 1 and 2 (IRS-1/IRS-2) was studied in rat and human myoblasts, and adult rat cardiomyocytes.
Results: Steady-state insulin receptor binding affinity was slightly less with GLU compared with HI (relative binding affinity vs HI was ~0.70). IGF-1 receptor binding affinity was lower (4–5 fold) for GLU relative to HI, in contrast to Asp(B10), which showed significantly higher (4-fold) affinity relative to HI. GLU, Asp(B10) and HI showed similar insulin receptor association kinetics; however, Asp(B10) revealed an increased insulin receptor affinity. While GLU and HI were similar with respect to insulin receptor-mediated activation of phosphorylation, activation with Asp(B10) was different with a prolonged phosphorylation state of the insulin receptor, and presence of receptor substrates. HI and GLU exerted similar activation of IRS-2 in the three cell systems studied. Activation of IRS-1 was 6–10-fold lower with GLU relative to HI.
Conclusions: Unlike Asp(B10), the characteristics of GLU are close to that of HI with respect to insulin receptor binding and activation of initial signalling events. In addition, IGF-1 receptor binding affinity was much lower with GLU than with HI. These data support that the predicted metabolic potency of GLU is similar to that of HI, and that the predicted mitogenic potential of GLU is not increased compared with HI.
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Insulin restores changes of skeletal muscles induced by diabetes mellitus

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Background and Aims: Diabetic neuromyopathy is charactized with muscle weakness and muscle wasting. Skeletal muscle requires insulin for normal growth and development. It is composed of district fiber types with unique metabolic characteristics and heterogenity of insulin action (slow-contracting fibers, called type I, and three different types of fast-contracting fibers, called IIA, IIX and IIB muscle fibers). It has been shown that progression of diabetes mellitus is concomitant with decrease of the regenerative capacity of skeletal muscle. The aim of present study was to investigate the influence of insulin during the long term diabetes on the process of slow and fast skeletal muscle regeneration.
Materials and Methods: Two-months-old male Wistar rats were randomised to control group, untreated diabetic group and diabetic group treated with insulin. We used a single streptozotocin (STZ) 65 mg/kg with i.p. injection for inducing experimental diabetes. Muscle regeneration was induced by injection of local anesthetic, bupivacain in slow (m.soleus, SOL) and fast (m. extensor digitorum longus, EDL) skeletal muscles. Morphometric analysis (fibre cross areas and fibre type distribution) of skeletal muscles was performed at intervals of 10 days, 4 weeks and 8 weeks.
Results:
Soleus muscle.
Four weeks after the process of regeneration started, in control group fiber type distribution was similar to normal SOL without the regeneration. In contrast, in diabetic muscles after eight weeks of regeneration, the percentage of fiber types did not reach values of control group and normal SOL without the process of regeneration. In the diabetic group treated with insulin fiber type distribution after four weeks was similar to these in control group.
In diabetic soleus muscles, during all investigated intervals, the fibre cross areas of type I and IIA were significantly smaller than in normal muscle. In diabetic soleus muscle treated with insulin, the fibre cross areas of type I and type IIA were significantly higher than in diabetic muscles after 10 days of treatment, but this effect was diminished after 8 weeks.
Extensor digitorum longus
After 8 weeks of experiment control muscles were completely regenerated. In diabetic untreated muscles in all observed subgroups, fibers type distribution was changed. After 8 weeks of diabetes the percentage of type I and IIA fibers, which have predominant oxidative metabolism, was greater. The treatment with insulin prevented changes in fiber type distribution during all investigated periods.
In diabetic EDL muscle, the cross areas of all fibre types were significantly smaller than in control muscles with an exception of fibre type I after 10 days. In diabetic group treated with insulin the fibre cross areas were significantly higher, than in diabetic group during the all experimental periods.
Conclusion: Diabetes mellitus is associated with evident decrease of the regenerative capacity of skeletal muscle. Treatment with insulin in long-term diabetes mellitus can prevent atrophy of regenerative slow and fast skeletal muscles.
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The effect of insulin dose and level of glycaemia on catabolism in critically ill patients

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Background and Aims: Evidence suggests that tight glycaemic control in critically ill patients can improve morbidity and mortality. The mechanism(s) underlying its benefit remain speculative but might involve an amelioration of catabolism. This study was designed to differentiate the contribution of the insulin dose to the level of glycaemia achieved, on the catabolic response.
Materials and Methods: A prospective study was conducted in 16 critically ill patients. Subjects with diabetes mellitus, pancreatitis, or liver disease were excluded. Patients were studied on 2 occasions, 48 hours apart. The baseline study was within 36 hours of admission to the ICU with blood glucose at 7–9 mmol/L. Patients were then randomised to one of four groups: Variable insulin with plasma glucose 4–6 mmol/L (LILG); Variable insulin with plasma glucose 7–9 mmol/L (LIHG); High-dose insulin ($2\,mU \cdot kg^{-1} \cdot min^{-1}$ plus requirement from baseline) and variable dextrose to maintain glucose 4–6 mmol/L (HILG); High-dose insulin and variable dextrose to maintain glucose 7–9 mmol/L (HIHG). Glucose production rate (R_a) and leucine R_a (a measure of protein degradation) were measured with a 3-hour infusion of [6,6$^2H_2$]glucose and [1-^{13}C]leucine. Steady state sampling was performed at 150 to 180 mins. Endogenous glucose R_a was calculated by subtracting the dextrose infusion rate from total glucose R_a. Leucine oxidation rate (Ox) was calculated from CO_2 enrichment and CO_2 production rate. Non-oxidative leucine disposal (a measure of protein synthesis) was calculated as leucine R_a minus leucine Ox. Non-esterified fatty acid concentrations provide an estimate of lipolysis.
Results: Protein turnover data (mean ± SEM) was compared with 12 fasted age-matched controls. Glucose turnover data was compared to a separate control group of 8 subjects.

		Control (n=12)	LIHG (n=4)	LILG (n=4)	HIHG (n=4)	HILG (n=4)
Glucose (mmol/L)	Baseline	5.02 ± 0.07	7.17 ± 0.78	7.6 ± 1.24	9.03 ± 0.91	8.09 ± 0.73
	End		8.72 ± 0.66	5.70 ± 0.50	8.6 ± 0.46	5.12 ± 0.46
Endogenous Glucose R_a ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	Baseline	8.7 ± 0.39	0.87 ± 3.15*	−1.39 ± 4.32*	2.97 ± 0.92	4.01 ± 1.98
	End		3.81 ± 0.97*	2.92 ± 1.71*	2.69 ± 2.43	−5.34 ± 5.95
Glucose R_d ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	Baseline	8.7 ± 0.39	22.85 ± 3.49*	22.90 ± 5.74*	23.89 ± 3.30*	28.06 ± 3.86*
	End		25.77 ± 1.09	24.19 ± 4.75	43.86 ± 6.84	33.58 ± 4.21
Leucine R_a ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	Baseline	1.63 ± 0.12	2.21 ± 0.48	2.05 ± 0.36	1.64 ± 0.12	1.86 ± 0.1
	End		2.13 ± 0.32	2.19 ± 0.37	2.37 ± 0.5	2.31 ± 0.09
NOLD ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	Baseline	1.37 ± 0.42	1.68 ± 0.43	1.59 ± 0.24	1.25 ± 0.19	1.59 ± 0.12
	End		1.36 ± 0.14	1.62 ± 0.24	1.92 ± 0.45	1.78 ± 0.18
NEFA (mmol/L)	Baseline	0.71 ± 0.09	0.25 ± 0.15*	0.09 ± 0.03*	0.12 ± 0.05*	0.13 ± 0.08*
	End		0.13 ± 0.03	0.48 ± 0.42	0.10 ± 0.04	0.07 ± 0.02

*P<0.01 v control, p<0.05 v baseline

Conclusions: Amongst non-surgical ICU admissions, the use of insulin to achieve less-stringent glycaemic targets was able to suppress glucose R_a and lipolysis and increase glucose uptake. No further suppression of glucose Ra was found with high dose insulin or with tighter glycaemic control. Leucine R_a was not decreased, even by pharmacological doses of insulin, whereas glucose R_d was significantly increased in the HILG and HIHG groups. These results suggest that the use of insulin to achieve normoglycaemia in the critical care setting does not promote whole body protein anabolism.

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Increased action of pulsatile compared to non-pulsatile insulin delivery during a meal-like glucose exposure simulated by computerised infusion in healthy humans

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Background and Aims: Insulin is released in a pulsatile manner both during baseline conditions and during meal-challenge. The increase in postprandial insulin secretion is achieved almost exclusively by amplification of the insulin secretion burst mass. It has previously been demonstrated that pulsatile insulin has greater hypoglycemic effect than continuous delivery during baseline euglycaemic conditions. The aim of the present study was to examine the effect of pulsatile versus non-pulsatile insulin delivery during a meal-like iv-glucose challenge.
Materials and Methods: Ten healthy young subjects with no family history of diabetes mellitus were examined on two occasions. A pancreatic-pituitary clamp was maintained with somatostatin infusion and replacement of glucagon and growth hormone at baseline levels. During the first three hours on both study days, insulin was infused in a pulsatile manner at a rate of 0.1 mU/kg/min every ten minutes followed by a nine-minute pause. Hereafter glucose and insulin was infused by computer-controlled pumps for four hours in a manner mimicking the postprandial glucose- and insulin profiles. At one study day, insulin infusion was done in a continuous manner, while at the other study day this profile was converted into a pulsatile profile by a computerised pump coupled to the insulin pump. Blood samples were drawn every 15 to 30 minutes for the analysis of glucose, insulin, c-peptide, growth hormone, glucagon and free fatty acids. The hypoglycaemic effect of insulin was measured as the integrated area under the curve during the four-hour infusion period.
Results: Endogenous insulin release was suppressed, while glucagon and growth hormone was equally substituted at the two study-days. The mean insulin concentration measured as the integrated area under the curve was identical ($p>0.9$). The hypoglycaemic effect of insulin was significantly increased by 13% during pulsatile delivery as compared to continuous delivery ($p=0.015$). Likewise was the maximal glucose concentration significantly lower at the day of the pulsatile profile (9.9 ± 1.0 vs. 11.4 ± 2.3 mmol/L, $p=0.036$).
Conclusion: The pulsatile insulin release plays an important role in the postprandial glucose homeostasis. The disturbed insulin pulsatility in type 2 diabetes may contribute to the postprandial hyperglycaemia.

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Differential effects of insulin aspart and human regular insulin on body composition and memory in humans

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Background and Aims: Insulin has been shown to enter the cerebrospinal fluid compartment after intranasal administration without absorption into the blood stream. Moreover, intranasal administration of insulin for 8 weeks induced a significant reduction in body weight and body fat mass and an improvement in memory function in healthy subjects compared to placebo treatment. From clinical trials evidence exists, that weight gain is less pronounced during subcutaneous treatment with insulin aspart as compared to human regular insulin in patients with diabetes, which could reflect an altered central nervous effect of insulin aspart on body weight regulating centers within the brain. We evaluated the effects of intranasal administration of insulin aspart, human regular insulin and placebo during 8 weeks on body weight and body composition as well as on memory function in healthy subjects.
Materials and Methods: After a baseline phase of 2 weeks, 34 healthy men were treated with intranasal insulin aspart (4 × 40 IU/day, n=10), human regular insulin (4 × 40 IU/day, n=12) or placebo (n=12) in a double blind, between subject comparison for 8 weeks. Body weight, body composition (bioelectrical impedance analysis) and declarative memory (immediate and delayed recall of word lists) were determined every week during the baseline phase and during the 8 weeks of intranasal treatment. Statistical analyses based on analyses of covariance with the values of the baseline sessions serving as covariates.
Results: Blood glucose levels did not differ between the treatment conditions. At the end of the treatment phase subjects had significantly lost body

weight during intranasal treatment with insulin aspart (mean±SEM: -1.25 ± 0.52 kg, p<0.05) and regular insulin (-1.0 ± 0.63 kg, p<0.05) compared to the placebo condition. Loss in body fat mass was more pronounced during intranasal treatment with insulin aspart (-3.33 ± 0.91 kg) as compared to regular insulin (-1.38 ± 0.59 kg, p<0.06). Lean body mass remained unchanged during the placebo and regular insulin condition, but increased during treatment with insulin aspart (+2.49 ± 0.71 kg, p<0.009 vs. placebo, p<0.03 vs. regular insulin). Bioelectrical impedance analysis revealed an increase in body cell mass, mainly reflecting muscle cell mass, in the insulin aspart condition (+2.04 ± 0.80 kg) compared to the regular insulin (-0.02 ± 0.46 kg, p<0.02) and the placebo condition (-0.09 ± 0.43 kg, p<0.02).
Delayed recall of words significantly improved after 8 weeks of intranasal administration of insulin aspart (remembered words: 6.06 ± 0.96) compared to placebo (2.17 ± 0.75, p<0.03) and regular insulin (3.47 ± 0.56, p<0.09). This differential effect was most pronounced for emotional words (insulin aspart: 2.47 ± 0.48, regular insulin: 1.27 ± 0.58, p<0.03, placebo: 0.64 ± 0.24, p<0.01).
Conclusion: Results indicate a direct action of prolonged intranasal administration of insulin on brain functions, and confirm findings of foregoing studies, showing a decrease in body fat mass and an improvement in memory after intranasal insulin in humans. The results provide first evidence, that insulin aspart exerts stronger effects on these brain functions compared to regular insulin. This differential effect could contribute to the less pronounced weight gain observed in clinical trails during subcutaneous treatment with insulin aspart compared to human regular insulin in patients with diabetes.

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Insulin action, inflammation and metabolic syndrome

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Acute inhibition of lipolysis does not affect post-prandial suppression of endogenous glucose production

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Background and Aims: To test the hypothesis that intra-hepatic availability of fatty acid could modify the rate of suppression of endogenous glucose production (EGP).
Materials and Methods: Acipimox or placebo was administered before and during a test meal in 11 healthy subjects. A modified isotopic methodology was used to measure EGP. ^{1}H MR spectroscopic measurement of hepatic triglyceride stores was undertaken.
Results: Acipimox suppressed plasma FFA markedly before the meal (0.05 ± 0.01 mmol/l at -10 min, p=0) and throughout the post-prandial period (0.03 ± 0.01 mmol/l at 150 min). Mean peak plasma glucose was significantly lower after the meal on acipimox days (8.9 ± 0.4 vs. 10.1 ± 0.5 mmol/l p<0.01), as was mean peak serum insulin (653.1 ± 99.9 vs. 909 ± 118 pmol/l; p<0.01). Fasting EGP was similar (11.15 ± 0.58 µmol/kg/min placebo vs. 11.17 ± 0.89 mg/kg/min acipimox). The rate of suppression of EGP following the meal was almost identical on the two test days (4.36 ± 1.52 vs. 3.69 ± 1.21 µmol /kg/min at 40 min). There was a significant negative correlation between the acipimox-induced decrease in peak plasma glucose and liver triglyceride content (r = -0.827, p=0.002), suggesting that in the presence of low levels of liver fat inhibition of lipolysis was able to affect glucose homeostasis.
Conclusion: Acute pharmacological sequestration of fatty acids in triglyceride stores dramatically improves postprandial glucose homeostasis without effect on the immediate postprandial suppression of EGP.
Support: Wellcome Trust

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Postprandial interstitial insulin and glycerol concentrations in the adipose tissue after nateglinide or placebo – insulin action in vivo is primarily dependent on cellular insulin sensitivity

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Background and Aims: It has previously been suggested that there is an endothelial barrier for the transport of insulin to the target cells, contributing to insulin resistance. The aim of the current study was to characterize the interstitial insulin concentration (I-Insulin) in human subcutaneous adipose tissue during physiological hyperinsulinemia in a group of healthy subjects with propensity to develop type 2 diabetes. In parallel, the insulin action on lipolysis, as measured by the subcutaneous glycerol concentration (I-Glycerol), was studied.
Material and Methods: Eight healthy first-degree relatives of type 2 diabetes patients were recruited (Gender: 5M/3F; Age: 48 ± 2 years (Mean±SE); BMI: 26.9 ± 0.9 kg/m^2; W/H-ratio: 0.91 ± 02). I-Insulin and I-Glycerol were measured by abdominal subcutaneous microdialysis. The insulin concentration in the microdialysis samples was analysed by an ultrasensitive ELISA method. Adipose tissue blood flow (ATBF) was studied by ^{133}Xenon-clearance. To achieve physiological hyperinsulinemia the measurements were performed after a test meal, with or without preceding administration of the insulin secretagogue nateglinide (Starlix®, 120 mg), in a double-blind cross-over design.
Results: I-Insulin increased after the test meal, and this response was more prominent after intake of nateglinide (Nate) as compared to placebo (Plac) (I-Insulin IAUC Nate 7612 ± 3032 vs Plac 4682 ± 2613 pmol x l^{-1} x min; p<0.05, Mean±SE). However, the postprandial I-Insulin$_{max}$ / P-Insulin$_{max}$

ratio was similar on the two test days (Nate: I-Insulin$_{max}$ 213 ± 62 vs P-Insulin$_{max}$ 501 ± 92 pmol/l, I/P-ratio: 0.38 ± 0.06; Plac: I-Insulin$_{max}$ 159 ± 39 vs P-Insulin$_{max}$ 410 ± 74 pmol/l, I/P-ratio: 0.36 ± 0.05). The delay of the insulin transport from the plasma to the interstitium was ≈13 min at the Plac day ($p<0.05$) and ≈14 min at the Nate day (n.s.). The ATBF response was similar at the two study days (ATBF IAUC Nate 157 ± 68 vs Plac 122 ± 97 ml/100 g; n.s.). Furthermore, there was no difference in time of onset of insulin action in situ, or responsiveness, measured as antilipolytic effect when comparing the Plac and Nate days. The half-maximal antilipolytic response in the adipose tissue after the meal was seen 15–20 min after the start of the meal, corresponding to an I-Insulin level of ≈90 pmol/l.

Conclusion: Microdialysis can now be used to measure the I-Insulin in human adipose tissue following a mixed meal. The postprandial I-Insulin is lower than the P-Insulin level, and there is a delay in the transport to the interstitium. However, the similar I/P-ratios obtained at the two different circulating insulin levels following nateglinide and placebo, support the concept that subcutaneous insulin resistance is mainly due to defects at the cellular level and not due to an endothelial barrier for insulin delivery to the cells.

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Fatty liver disease is associated with both endotoxemia and sub-clinical inflammation prior to the onset of type 2 diabetes

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Obesity-mediated diabetes is associated with insulin resistance/hyperinsulinaemia and chronic sub-clinical inflammation. Recent studies have highlighted fatty liver as a risk factor for diabetes. Fatty liver disease (FLD) progresses through fatty liver, necro-inflammation and fat with fibrosis. Hepatic steatosis *per se* can lead to hepatic insulin resistance/hyperinsulinaemia, which may contribute significantly to the development of T2DM. Previous *in vitro* studies have indicated that the liver may initiate an innate immune response, possibly due to increased gut permeability via hyperinsulinaemia, resulting in increased bacterial absorption. However, no study has confirmed whether there is a causal link between increased endotoxin levels and the progression of FLD, *in vivo*. Therefore in this study we (a) investigated the impact of FLD (Age: 36.8(mean±SD)11.5yrs) BMI: 26.5 ± 4.4 kg/m^2, n=80) and diabetic status compared to case controls (CC) (Age: 38.9 ± 12.4yrs; BMI: 26.5 ± 4.4 kg/m^2; n=35; LERC approved), (b) assessed inflammatory markers, including soluble CD14 (sCD14), resistin and the anti-inflammatory cytokine, adiponectin. The findings from this study showed that endotoxin levels were significantly higher in patients with FLD compared with CC (FLD:11.06(mean±SE) 0.96EU/mL; CC:5.27 ± 0.45EU/mL, $p<0.01$). FLD alone produced comparable endotoxin levels to T2DM (FL:T2DM:10.82 ± 1.15EU/mL; non-diabetic: 11.16 ± 0.90EU/mL). Furthermore, sCD14 and resistin were both raised in FLD subjects ($p<0.05$) whilst adiponectin was reduced in FLD compared with CC (FLD:11.63 ± 0.60 mg/mL vs CC:17.06 ± 1.74 mg/mL, p=0.006). In summary, our results suggest that FLD is associated with a 1.5 fold increase in endotoxemia levels compared with CC. FLD is also associated with hyperinsulinaemia, which may mediate increased gut permeability and hence endotoxemia, prior to the onset of T2DM. As such, impaired liver metabolism may interfere with portal circulation, metabolic clearance and increase the pathogenic risk of T2DM and cardiovascular disease.

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Proinflammatory cytokines and insulin resistance in non-diabetic patients with hepatitis C virus infection: a case-control study

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Background and Aims: We have previously shown that chronic hepatitis C virus (HCV) infection is associated with an increased risk of developing type 2 diabetes mellitus (DM). This relationship is already present in anti-HCV positive patients with chronic hepatitis and normal transaminases. The specific mechanisms for this association are not fully understood but it is possible that an increase in insulin resistance induced by proinflammatory cytokines plays an important role in the development of diabetes in HCV infected patients. To shed light on this issue a case-control study in non-diabetic patients with chronic hepatitis was performed.

Materials and Methods: From January to March 2004, a total of 14 consecutive non-diabetic (FBG <110 mg/dl) patients with chronic hepatitis other than HCV infection referred to the outpatient Liver Unit were recruited. Each patient of this group was carefully matched with two anti-HCV RNA (+) patients (n=28) for the following variables: age, sex, body mass index, waist-to-hip ratio and family history of DM. Chronic hepatitis was histologically confirmed by percutaneous liver biopsy. The pro-inflammatory cytokines (TNF-α, sTNFR1, sTNFR2 and IL-6) were assessed by ELISA. Insulin resistance was determined by the homeostasis model assessment (HOMA). Beta-cell function was evaluated by calculating the area under curve (AUC) of insulin and C-peptide after both a 200 ml standard food intake and intravenous administration of 1 mg of glucagon. Comparisons between groups [anti-HCV (+) and anti-HCV (-)] were made using the Student's t test for continuous variables and the chi square test for categorical variables.

Results: Anti-HCV (+) patients had higher proinflammatory cytokines than anti-HCV (-) patients (TNF-α: 6.47 ± 3.44 vs. 2.71 ± 3.06 pg/ml, p= 0.007; sTNFR1: 173.95 ± 34.73 vs. 149.41 ± 27.06 pg/ml, p=0.04; sTNFR2: 299.91 ± 78.55 vs. 241.75 ± 81.55 pg/ml, p=0.046 and IL-6: 3.78 ± 1.45 vs. 2.07 ± 1.02 pg/ml, p=0.002). In addition, baseline serum insulin, C-peptide and HOMA were also higher in anti-HCV (+) in comparison with anti-HCV (-) patients (serum insulin: 19.04 ± 9.43 mU/l vs. 11.04 ± 6.96 mU/l, p= 0.008; C peptide: 3.80 ng/ml (1.20–8.00) vs. 2.25 ng/ml(1.70–7.10), p=0.038; HOMA: 4.47 ± 2.23 vs. 2.58 ± 1.74, p=0.009). The AUC of both insulin and C-peptide after standard food intake and an intravenous glucagon test were similar in both groups. However, HCV (+) patients showed higher insulin and C-peptide peaks 5 minutes after glucagon test as well as higher C-peptide at 90, 120 and 180 minutes after standard food intake.

Conclusion: We conclude that non-diabetic HCV (+) patients are more insulin resistant than HCV (-) patients, proinflammatory cytokines being a primary contributor. Our results suggest that proinflammatory cytokines play a key role in the development of insulin resistance and type 2 diabetes in HCV infected patients.

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Free fatty acid-induced metabolic syndrome in obese African Americans

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Background and Aims: The metabolic syndrome, also known as the dysmetabolic syndrome, is associated with increased cardiovascular morbidity and mortality. An increase in free fatty acids (FFA) is a leading candidate in the pathogenesis of insulin resistance, diabetes, and hypertension and thus is a likely factor in the development of the metabolic syndrome. The effects of FFA on insulin action are well established; however, the effects on blood pressure (BP) and endothelial function are not very well understood. Accordingly, we evaluated the effects of increased FFA on BP, endothelial function, and insulin sensitivity.

Materials and Methods: Endothelial function was assessed directly by measuring brachial artery flow-mediated dilatation (FMD), and indirectly by changes on vascular inflammatory markers. We studied 18 obese African American (AA) normotensive diabetic subjects (age: 40 ± 2 yr, 11M/7F, BMI 37 ± 1 kg/m^2), and 6 obese AA normotensive nondiabetic subjects (age: 42 ± 1 years, 3F/3M, BMI 32 ± 1 kg/m^2). Within 2 weeks, they received both a 48 hr infusion of Intralipid (20%, 40 ml/hr) plus heparin (250 units/h) and a 48 hr infusion of normal saline (40 ml/hr) plus heparin.

Results: As expected, Intralipid infusion increased FFA levels from a baseline of 0.5 ± 0.47 mmol/L to 1.25 ± 0.9 mmol/L (150% increase). It also increased insulin resistance as indicated by a >3-fold rise in insulin and C-peptide levels without significant changes in glucose concentration. At both 8 and 24 hr, Intralipid infusion resulted in a significant BP rise from baseline of 14 mm Hg in SBP and 8 mm Hg in DBP. Compared to baseline, Intralipid infusion reduced FMD by 14%, whereas saline increased FMD by 18%. Levels of TNF-α peaked at 18 h of infusion and remained elevated, while levels of C-reactive protein continued to increase throughout the infusion with a peak increase of 93% at 44-h. Levels of vascular cell adhesion molecules (s-ICAM) and monocyte chemotactic protein-1 also increased during lipid infusion.

Conclusion: Increased FFA in obese AA subjects with and without diabetes results in the development of an "acute metabolic syndrome" characterized by hypertension and insulin resistance, as well as, endothelial dysfunction and inflammation. The FFA-induced acute metabolic syndrome constitutes a useful model to examine disease mechanisms and test therapeutic interventions to correct underlying disorders associated with the components of the metabolic syndrome.

Support: GlaxoSmithKline

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Are the patients with metabolic syndrome as defined by NCEP-ATP III criteria truely insulin resistant? from Chungjoo Insulin Resistance syndrome Cohort (CIRC) Study

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Objectives: To evaluate how well metabolic syndrome defined by NCEP-ATP III criteria can reflect insulin resistance and what factor best determines insulin resistance.
Methods: We performed cross-sectional study including 5330 participants (2197 males, 3133 females), over 40 years old in Chungjoo Insulin Resistance syndrome Cohort. Metabolic syndrome was defined by the NCEP-ATP III criteria and insulin resistance was analyzed by HOMA-IR. All participants were subdivided into four groups (quartile) by their HOMA-IR levels, and insulin resistance (IR) group was defined as the highest quartile group. Diagnostic accuracy for IR was evaluated using sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) and area under the curve (AUC) of ROC for each of the five parameters of the NCEP-ATP III criteria plus the presence of metabolic syndrome itself.
Results: The number of subjects with metabolic syndrome as defined by NCEP-ATP III and IR group was 1487 and 1165, respectively. Only 35.5% of the subjects with metabolic syndrome as defined by NCEP-ATP III belonged to the IR group. On the other hand, 54.5% of the IR group was not diagnosed as metabolic syndrome (sensitivity; 56.8%, specificity 67.9%, PPV; 33.1%, NPV; 84.9%). Fasting plasma glucose and body mass index (BMI) had the somewhat higher diagnostic accuracy value (AUC of fasting plasma glucose; 0.704, AUC of BMI; 0.659) than others. Stepwise multiple logistic regression analysis was also done to elucidating the best factor for determining the presence of IR and the parameter that best reflected the IR group was the BMI, for both male and female.
Conclusion: Metabolic syndrome as diagnosed by the NCEP-ATP III does not equate insulin resistance status, and BMI was the best determining factor for insulin resistance in adults over 40 years old in the CIRC study.

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Growth hormone and IGFBP-3 but not IGF-1 are independent predictors for insulin sensitivity in healthy subjects: on the role of hGH in the metabolic syndrome

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Background and Aims: Insulin resistance (IR) and obesity are very common metabolic abnormalities, which are often associated with reduced hGH secretion. GH reduces intraabdominal fat and its deficiency may contribute to the metabolic syndrome. IGF-1 improves IR but IGFBPs have a potential role in regulating its bioactivity although only little information exists. Glucose and insulin acutely suppress hGH secretion. We postulate that the elevated insulin levels in IR may persistently suppress hGH provided its regulation remains insulin sensitive. We therefore tested the sensitivity of the hGH axis to insulin suppression.
Materials and Methods: Seventy healthy subjects underwent assessment of fasting total IGF-1, fasting IGFBP-3 and fasting and post-OGTT GH and insulin levels (26 men and 44 women; age 25–73 years; BMI 28.9 ± 0.7 kg/m^2). IGF-I levels in all subjects were within their age-appropriate normal ranges. Insulin sensitivity was estimated by calculating the homeostasis model assessment (HOMA) scores, the quantitative insulin sensitivity check index (QUICKI) and the composite insulin sensitivity index (ISI). Bivariate and multiple linear regression analyses were performed to estimate the effects of different predictors of insulin sensitivity.
Results: In healthy subjects all three indices for IR are well correlated (r=0,68–0,88, p<0.0001). All subjects show a nadir GH <1 µg/l during OGTT (mean±SEM: 0.133 ± 0.0176). On bivariate linear regression, IGFBP-3 and log-transformed nadir GH levels but not total IGF-1 levels are significant predictors for insulin sensitivity (r=0.354–0.372, p<0.005 and r=0.277–0.373, P<0.05 respectively) even after correction for total IGF-I levels. We explain the positive correlation between IGFBP-3 and IR through its ability to reduce free IGF-1 levels since a negative correlation between IGF-1/BP-3-ratio, as an indicator for free IGF-1 levels, with IR is indicated (r=0.271–0.289, p<0.05). Moreover, the negative correlation between nadir GH and IR may be due to a higher insulin/glucose-induced GH suppression after glucose ingestion since a negative correlation between nadir GH and peak post-glucose insulin level is seen (r=0.313, p=0.008).
Conclusion: Our data indicate that hGH secretion remains sensitive to insulin in insulin resistance. IGFBP-3 and nadir GH are more predictive than total IGF-1 of insulin sensitivity. We show for the first time that higher IGFBP-3 and lower free IGF-1 estimated by IGF-1/BP-3 ratio are associated with a higher insulin resistance indicating a role of endogenous IGF-1 in glucose homeostasis in healthy individuals.

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BMI and ratio of triglycerides to HDL are most predictive of insulin resistance in a drug naive population with type 2 diabetes

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Background and aims: Insulin resistance (IR) is recognised as one of the key abnormalities contributing to the development of type 2 diabetes (T2D). Furthermore identifying IR in T2D patients may aid physicians in the selection of the most appropriate antidiabetic therapy. The aim of this analysis was to identify severe IR patients using easily assessed clinical parameters and insulin sensitivity (%S) as determined by the homeostatic model assessment (HOMA-S) in recently diagnosed (≤3 years duration) drug-naive patients with T2D.
Methods: The clinical measures included were age, gender, race, blood pressure, serum triglycerides (TG), HDL-cholesterol, ratio of TG:HDL, waist-hip ratio, waist and hip circumference, and urinary albumin:creatinine ratio. These data were acquired from baseline data in drug-naïve T2D patients recruited into the ADOPT (A Diabetes Outcome Prevention Trial) study (n=4092). For predicting IR, four different methods were used: neural network, CART (Classification and Regression Tree), multiple linear regression and random forest.
Results: All statistical methods were congruent in identifying BMI and TG:HDL ratio as the two factors that consistently predicted incident Insulin Sensitivity with R^2 =0.26 (Table).

Importance Order	Neural Networks	CART	Linear Regression	Random Forest
1	BMI	BMI	BMI	BMI
2	TG:HDL	TG:HDL	TG:HDL	TG:HDL
3	Waist Cir.		Waist Cir.	Waist Cir.
4	Race		Gender	Hip Cir.

Furthermore, a simple algorithm that utilised BMI and TG:HDL ratio, was successful in predicting severe IR (defined as %HOMA-S <30) with a 75% sensitivity & 65% specificity in the following groups:
(i) Non-obese (BMI<29 kg/m^2) with TG:HDL ratio >8 or
(ii) Overweight or obese (BMI 29–35 kg/m^2) with TG:HDL ratio> 3 or
(iii) Severe obesity (BMI>35 kg/m^2) with any TG:HDL ratio
Conclusions: In a population of recently diagnosed patients with T2DM, BMI and TG:HDL ratio correlate most strongly with insulin resistance. A simple algorithm comprising of these two easily assessable clinical factors can be used to identify severe insulin resistance and could be of use to physicians for selecting the most appropriate treatment regimens for patients.

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Relationship between insulin sensitivity and carotid artery intima-media thickness in healthy subjects: the RISC Study

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Background and Aims: Insulin resistance is thought to favour the development of atherosclerosis. The available evidence is, however, inconclusive, possibly because insulin sensitivity has only been inferred from surrogate measurements in cohorts of subjects with multiple cardiovascular risk factors (most of which bear some relation to insulin resistance).
Materials and Methods: In the RISC (Relationship between Insulin Sensitivity and Cardiovascular disease) study, 1,500 healthy subjects aged 30–60 yrs were recruited at 19 centres in 14 European countries and are being fol-

lowed up. In addition to anthropometrics and lifestyle variables, each subject received a standard OGTT, a euglycaemic hyperinsulinaemic (40 mU/m^2 per min) clamp, and an ultrasound evaluation of carotid artery (CA) intima-media thickness (IMT). IMT was measured in common CA, bulb and internal CA on both sides and the overall average was calculated (CA-IMT). We report here on the subjects who completed the baseline studies and for whom the following variables were available: fasting and postglucose plasma glucose (f-PG and 2 h-PG) and insulin (f-PI and 2 h-PI) concentrations, blood pressure (SBP and DBP), insulin sensitivity (M value expressed as µmol/min/kg$_{ffm}$), serum total, HDL and LDL cholesterol (Chol) and plasma triglycerides (TG).
Results: The study cohort consists of 1,157 subjects (645 women and 512 men) aged 44 ± 8 yrs (mean±SD) [range 30–60], with a mean body mass index (BMI) of 26.2 ± 3.4 kg/m^2 [range 17–42]. SBP was 117 ± 12 mmHg, DBP was 74 ± 8 mmHg, f-PG and 2 h-PG were 5.1 ± 0.6 and 5.8 ± 1.5 mmol/l, f-PI and 2 h-PI were 34 ± 19 and 213 ± 248 pmol/l, total, HDL and LDL-Chol were 4.86 ± 0.88, 1.43 ± 0.38 and 2.93 ± 0.80 mmol/l, respectively, serum TG was 1.08 ± 0.71 mmol/l. The M value averaged 57 ± 23 µmol/min/kg$_{ffm}$ ranging from 12 to 153 µmol/min/kg$_{ffm}$. CA-IMT averaged 665 ± 103 µm ranging from 444 to 1,006 µm. In univariate analysis (only adjusting by centre), M was inversely associated with mean CA-IMT (-4 ± 1 µm per each 10 µmol/min/kg$_{ffm}$, p<0.002). No association was observed between CA-IMT and 2 h-PI, whereas CA-IMT was positively related to 2 h-PG (+5 ± 2 µm per mmol/l, p<0.03). When adjusted for the effect of gender (+16 ± 3 µm in men, p<0.00001), age (+50 ± 34 µm per each 10 years, p<0.0001), BMI (+2 ± 1 µm per unit, p=0.03), LDL-Chol (+20 ± 4 µm per mmol/l, p<0.00001) and SBP (+9 ± 2 µm per each 10 mmHg, p=0.0004), ie, the main determinants of IMT (r^2=0.31), both M and 2 h-PG lost their association with CA-IMT (p=0.36).
Conclusion: In cross-sectional observations in a low-risk population, insulin resistance is not an independent determinant of intima-media thickness in the carotid artery. The follow-up phase of RISC will conclusively test whether insulin resistance per se is atherogenic.
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Insulin secretion in prediabetes and unique diabetes subtypes

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Increased secretion of incretin hormones and insulin in patients with chronic pancreatitis and exocrine pancreatic insufficiency following enzyme substitution
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Background and Aims: We aimed to investigate how the digestion and absorption of nutrients affect the secretion of the insulinotropic incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), and to evaluate the effect of pancreatic enzyme substitution (PES) on insulin secretion in patients with chronic pancreatitis (CP) and pancreatic exocrine insufficiency (PEI).
Materials and Methods: Eight male patients with CP and PEI (Age: 57.0 (48–66) years; BMI: 21.2 (15.2–27.4) kg/m^2; HbA_{1C}: 6.2 (5.1–7.7) % (mean (range)) were studied. Blood was sampled on two separate days over 4 hours after ingestion of a liquid meal with and without PES. A group of 8 healthy subjects matched for gender, age and BMI were used as controls. Sampling of triglycerides was used to evaluate the level of nutrient absorption.
Results: No difference in plasma glucose could be found (406 vs. 425 mM x 4 h) on the two days (p = 0.755). The secretion of GLP-1 was significantly higher after PES (7.7 vs. 5.3 mM x 4 h (p = 0.010)) like the secretion of GIP (32.7 vs. 21.2 mM x 4 h (p = 0.010)). Concurrently both plasma insulin and C-peptide increased after PES (18 vs. 14 mM x 4 h (p = 0.019) and 237 vs. 200 mM x 4 h (p = 0.005), respectively).
Conclusion: These results suggest that the secretion of GLP-1 and GIP is dependent on the digestion and subsequent absorption of nutrients in the small intestine, and that PES via increased secretion of incretin hormones has a positive effect on the secretion of insulin in patients with CP and PEI.
Support: The Danish Diabetes Association

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Preserved incretin response in subjects with maternally inherited diabetes and deafness (MIDD)
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Background and Aim: The 3243A>GmtDNA may lead to Maternally Inherited Diabetes and Deafness (MIDD) and is the most common cause of mitochondrial diabetes. The mutation may cause impaired ATP production which is believed to partly account for the impaired insulin secretion observed in patients with MIDD.
The two intestinal insulin-stimulating hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are most important to the insulin response when glucose is ingested and in patients with type 2 diabetes the secretion of GLP-1 is reduced leading to impaired incretin effect.
We therefore aimed to evaluate the incretin response to OGTT in mtDNA A3243G mutation positive subjects both with and without diabetes
Material and methods: Eighteen 3243A>G mtDNA individuals from four pedigrees: (Mean, range) Age: 41 years (18–62), BMI: 21.0 (18–33). Male: female-ratio 7:11. Glycemic status: normal glucose tolerance (NGT) n = 4, impaired glucose tolerance (IGT) n =7, diabetes mellitus (DM) n = 7. The 3243A>G mtDNA subjects and a control group matched by gender, age and BMI underwent a three hour oral glucose tolerance test. P-GLP-1, GIP and glucagon was measured at time = -15,-10,-5, 0, +15, 30, 45, 60, 90, 120, 180 minutes.

Results: No significant differences in the incretin responses between NGT, IGT, DM 3243A>G subjects and controls could be identified. The result showed large variance within the groups. Futher there was no difference in fasting p-glucagon.
Conclusion: In present study 3243A>G mtDNA mutation positive subjects with NGT, IGT and DM seems to have preserved incretin response when ingesting glucose. Thus impaired GLP-1 and GIP secretion does not seem to contribute to the insulin secretion defect observed with mitochondrial diabetes

Incretin response in NGT and 3243 mtDNA positive subjects

	Controls Median (Range)	$NGT_{3243A>G}$ Median (Range)	$IGT_{3243A>G}$ Median (Range)	$DM_{3243A>G}$ Median (Range)	Levels of significance
Fasting p-GIP (pmol/L)	3.7 (2–25)	2,2 (2–12)	2,6 (2–11)	3.9 (2–37)	NS
Fasting p-GLP-1 (pmol/L)	7.5 (2–25)	5.5 (2–20)	5 (2–20)	4 (2–46)	NS
$AUC_{GIP,180\ min}$	17.7×10^3 (4.4–37.3)	9.6×10^3 (7.3–23.4)	13.2×10^3 (3.0–27.9)	16.64×10^3 (7.0–43.7)	NS
$AUC_{GLP\text{-}1,180\ min}$	5.6×10^3 (3.2–13.8)	5.1×10^3 (3.2–7.9)	5.2×10^3 (3.1–10.8)	6.4×10^3 (4.2–13.0)	NS
Fasting p-glucagon (pmol/L)	7 (4–15)	9.5 (6–13)	9 (5–12)	7 (4–15)	NS

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Deficient insulin secretion in response to glucose but not to arginine in patients with type 1B diabetes during remission
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Background and Aims: Idiopathic type 1 or type 1B diabetes is a syndrome characterized by acute hyperglycemic and ketotic onset in the absence of autoimmune markers of type 1 diabetes, and a subsequent prolonged non-insulin dependent near-normoglycemic remission in more than 50% of the patients of unclear etiopathogenic mechanisms. The objective of this study was to characterize β cell function during remission.
Materials and Methods: We studied insulin action, insulin secretion, and body composition in 14 type 1B diabetic patients (13M/1F, age = 45 ± 8 (SD) yr, HbA1c = 6.1 ± 0.8%, BMI = 29.8 ± 4.2 kg/m^2) in remission since at least 3 months.They were compared to 13 (11M/2F) normoglycemic control subjects (age = 43 ± 9yr, BMI = 24.8 ± 2.4 kg/m^2). All the patients and control subjects were of Sub Saharan African origin. Type 1B diabetes was considered for patients with hyperglycaemia and ketosis at onset, in the absence of autoimmune markers of type 1 diabetes, and subsequent remission. We measured insulin sensitivity by a 80 mUI/m^2/min euglycemic hyperinsulinemic clamp. We evaluated insulin secretion in response to 75 g oral glucose load, and to a 200-min graded intravenous glucose stimulation (4 consecutive steps ranging from 2 to 10 mg/kg/min). Following the 200-min graded glucose infusion, infusion rate was adjusted, with the aim to obtain ≅22 mmol/L blood glucose, and a bolus of 5g arginine was administered, with blood samples taken every minutes over 10 minutes to measure insulin secretion. Insulin secretion rate was estimated by C-peptide deconvolution analyses. Early insulin secretion in response to oral glucose was estimated using the insulinogenic index (30-minute incremental insulin/glucose concentrations). Results are expressed as mean±standard error. Changes in glucose, insulin and C-peptide levels over the clamp and the glucose infusion studies were analysed using one way repeated measures analysis of variance. Comparison between groups were performed using the non-parametric Wilcoxon matched pairs signed rank test.
Results: Whole body insulin sensitivity (M-value) was 6.9 ± 0.7 and 10.5 ± 1.1 mg/kg of fat-free mass/min in patients and controls respectively ($p < 0.01$). Early insulin secretion in response to oral glucose (insulinogenic index) was 5.7 ± 1.1 vs 13.2 ± 2.4 mUI/mmol in patients compared to controls ($p < 0.01$). The graded glucose infusion yielded a 49% lower C-peptide concentrations in patients compared to controls (1.88 ± 0.30 vs 3.67 ± 0.47 pmol/ml, $p < 0.01$) at the highest IV glucose stimulation, while acute insulin response to combined arginine + glucose was 11.6 ± 2.5 vs 14.4 ± 3.1 pmol in patients vs controls equivalent to 19% reduction (NS).
Conclusion: Despite a marked reduction of insulin secretion in response to oral and intravenous glucose, little changes of the response to the combined glucose + arginine stimulation were observed in patients with type 1B diabetes during remission. Since the insulin secretory response to the combined glucose and arginine stimulation is one of the best available estimates of β cell mass *in vivo*, our results suggest that β cell dysfunction might predominates over β cell destruction in type 1B diabetes.
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Glucagon-like peptide-1 and β-cell function in subjects with normal or impaired glucose tolerance
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Background and Aims: The increased insulin secretion (ISR) after oral as compared to intravenous (IV) glucose is attributed to the actions of incretin hormones. Aim of this study was to evaluate the relationship between GLP-1 release and β-cell function at matched glycaemic levels in subjects with normal (NGT) or impaired (IGT) glucose tolerance.
Materials and Methods: 12 NGT subjects (5M/7F; 45 ± 4 years; BMI: 25.5 ± 1.0 kg/m^2) and 10 IGT subjects (2M/8F; 54 ± 3 years; BMI: 30.8 ± 1.2 kg/m^2) received a 3-hr OGTT (75 g) with timed measurements of plasma glucose, insulin and C-peptide concentrations. On a different day, the plasma glucose profile was reproduced by an IV glucose infusion. Insulin secretion was reconstructed by C-peptide deconvolution; a mathematical model was used to derive parameters of β-cell function (glucose sensitivity, ie, the slope of the dose-response relating glucose concentration to ISR; rate sensitivity, ie, a marker of early ISR; potentiation factor, an index of relative ISR potentiation due to various factors).
Results: As expected, total ISR (NGT+IGT together) was higher during oral than IV glucose (57 ± 5 vs 39 ± 4 nmol/m^2, $p<0.0001$) as was the plasma total GLP-1 response (area-under-curve = 5.3 ± 0.3 vs 3.4 ± 0.2 nM.3 h, $p<0.0001$). Both β-cell glucose sensitivity (119 ± 17 vs 70 ± 12 pmol/min.m2, $p=0.0004$) and rate sensitivity (1.3 ± 0.2 vs 0.7 ± 0.2 nmol/m^2.mM, $p=0.001$) were markedly higher following oral than IV glucose. In the whole group, the ISR parameters obtained from the oral and IV tests were correlated ($r=0.50–0.86$, $p<0.05$ or less). When comparing NGT with IGT subjects, the latter had worse β-cell glucose sensitivity than the former on both oral (75 ± 14 vs 155 ± 25 pmol/min.m^2, $p=0.001$) and IV glucose (39 ± 5 vs 96 ± 19 pmol/min.m^2, $p=0.002$), and a lower GLP-1 response to oral glucose (4.4 ± 0.4 vs 6.0 ± 0.4 pM.3 h, $p=0.02$). Furthermore, in either group or test or in the whole data set there was little quantitative relationship between total GLP-1 response and total insulin output or the β-cell function parameters. The model-derived potentiation factor did not differ either by test (OGTT vs IV) or by group (NGT vs IGT), suggesting that this parameter is related to exposure to hyperglycemia rather than to incretins.
Conclusion: The incretin effect, ie, the difference in β-cell response between oral and IV glucose, comprises both total ISR, glucose sensitivity and rate sensitivity. The incretin effect is largely preserved in IGT subjects. The changes in total GLP-1 response to glucose are insufficient to quantitatively explain the incretin effect in either NGT or IGT subjects.

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Metabolic and hormonal circadian rhythms in subjects with impaired glucose tolerance
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Background and Aims: The circadian rhythm (CR) of glucose tolerance (GT) is characterized by being greater in the morning than in the afternoon, called *afternoon diabetes*. This decreased GT in the end of day is due to a lower insulin (INS) secretion and INS sensitivity with decreased glucose (G) disposal. In diabetic subjects there are evidences for an inversion of this CR of GT. Impaired GT (IGT) is considered a precursor stage of *diabetes mellitus* in genetically predisposed individuals. It is important to diagnose and treat IGT people because they have increased risk for developing cardiovascular diseases. There are few studies about the CR of GT in these people. The aim of this study was to evaluate the CR of G and related hormones in subjects with IGT comparatively to others with normal GT (NGT).
Subjects and Methods: Individuals of both groups (IGT and NGT) were healthy, active, and free of any medication. They were studied under controlled conditions during 24 h that included a diurnal period with three basic meals (total energy intake of 2000 and 2500 kcal for women and men, respectively), light physical activities, and sleep night. During 24 h we took

frequent blood samples for measurements of G by Glucose oxidase (Beckman Ins.); specific INS and intact proinsulin (PROINS) by ELISA (Dako Diag. Ltda.); Cortisol (C) and growth hormone (HGH) by radioimunoassay (RIA - DPC Diag. Products Corp.); and non-esterified fatty acids (NEFA) by enzymatic colorimetric (WAKO Chem. GmbH). Data are presented as mean±SD, or median±interquartile semirange. Statistical significance was determined by Student-t and Mann-Whitney tests. P values of <0.05 were considered significant.
Results: We studied 15 (13F/2M) IGT individuals and 18 (14F/4M) NGT individuals with similar age, body mass index, and waist-hip ratio ($p>0.05$). Before the study both groups differed in relation to basal G (IGT: 100 ± 10 mg/dL; NGT: 90 ± 10 mg/dL, $p<0.05$) and 2 h-plasma G after an oral G load (75g) [IGT: 165 ± 14 mg/dL; NGT: 101 ± 19 mg/dL, $p< 0.001$]. IGT subjects'glucose total area under the curve (AUC_G) was significantly greater than that of NGT subjects (AUC_G-IGT: 2529 ± 257 vs AUC_G-NGT: 2226 ± 166 mg/dL/h, $p< 0,01$). Glucose profile showed higher glucose values during the morning in the IGT group and during the evening in the NGT group. Plasma INS levels followed those of G in both groups and were not different between them (AUC_{INS}-IGT: 570 ± 153 vs AUC_{INS}-NGT: 575 ± 206 µUI/mL/h, $p>0.05$). Plasma PROINS concentrations were higher in the IGT subjects (AUC_{PROINS}-IGT: 282 ± 67 vs AUC_{PROINS}-NGT: 153 ± 90 pmoL/L/h, $p<0.05$). Both groups presented similar plasma C, HGH and NEFA levels (AUC_C-IGT: 166 ± 28 vs AUC_C-NGT: 171 ± 27 µg/dL/h; AUC_{HGH}-IGT: 36 ± 18 vs AUC_{HGH}-NGT: 46 ± 32 ng/mL/h; AUC_{NEFA}-IGT: 20 ± 12 vs AUC_{NEFA}-NGT: 33 ± 11 mmoL/L/h, ($p>0.05$, respectively). Both groups showed the habitual cortisol CR.
Conclusion: Our results suggest that IGT subjects already present inversion of the CR of GT associated with higher plasma PROINS levels. The change of CR of GT could not be explained by alteration of C or HGH secretion. The higher levels of plasma G associated with similar plasma INS levels and higher plasma PROINS levels in IGT group compared with NGT group suggest impaired β-cell function.
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Insulin resistance vs. impaired insulin secretion in patients treated with novel antipsychotic drugs
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Background and Aims: Risk of incident diabetes mellitus is threefold increased in patients treated for schizophrenic psychosis. In addition, considerable weight gain and diabetic ketoacidosis have been described, particularly in patitents treated with modern antipsychotic drugs with serotonin 5HT2A receptor antagonistic properties, such as olanzapine (OL) and risperidone (RI). So far, no cases have been reported under amisulpride (AM), a selective dopamine D2/D3 antagonist. Pathogenesis of antipsychotic-related diabetes is unclear.
Materials and Methods: A) Euglycemic and hyperinsulinmic clamp studies were performed in 10 healthy subjects after acute administration of the atypical antipsychotic drugs olanzapine 10 mg or amisulpride 400 mg vs. placebo and B) after acute administration of the selective 5HT2A antagonist ketanserin 40 mg vs. placebo in a double blind cross-over design. C) HOMA-CIGMA tests were performed in patients with schizophrenic psychosis prior to and after 3 weeks of treatment with olanzapine (n=8) or risperidone (n=7).
Results: A) C-peptide secretion during hyperglycemic clamp was stimulated by amisulpride compared to olanzapine or placebo (ANOVA p=0.043), while insulin sensitivity was unchanged. B) Insulin sensitivity was impaired after ketanserine compared to placebo (euglycemic clamp glucose disposal rate 7.7 ± 2.4 vs. 9.4 ± 3.6 mg/kgBW/min; p=0.030). C) Weight gain was significantly greater after treatment with olanzapine compared to risperidone (4.6 ± 2.0 vs. 1.7 ± 1.3 kg, p=0.034), while HOMA insulin sensitivity was significantly reduced after treatment with olanzapine (80 ± 28% of baseline), but unaffected after risperidone (120 ± 29%, interaction time*treatment p=0.034).
Conclusion: Antagonism at the serotonin 5HT2A receptor may impair insulin sensitivity. Along with weight gain, this mechanism may be one reason for the development of diabetes in patients treated with antipsychotic drugs. Dopamine D2D3 antagonism appears to facilitate insulin secretion and may partially explain the lower incidence of diabetes in patients treated with the antipsychotic drug amisulpride.
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Effect of age, gender and diabetes on metabolism

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Metabolic remodelling in pre-pubertal children – testing the lipid flux hypothesis
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Background and Aims: The behaviour of insulin resistance and its metabolic correlates in response to changes in body fat has not been well characterised in children. Frayn's lipid flux hypothesis proposes that adipose tissue buffers the flux of fatty acids in circulation by 'trapping' them and thus increasing triglyceride clearance. We tested this hypothesis by examining trends in adiposity, insulin resistance and metabolic risk variables in healthy pre-pubertal children.
Materials and Methods: EarlyBird is a non-intervention, prospective cohort study documenting physical, metabolic and lifestyle development in 300 healthy children from five years. Children were recruited in 2000/1 from a wide range of socioeconomic backgrounds. Data were obtained from 230 children (130 boys, 100 girls) who attended at 5, 6, 7 and 8 years. Measures included the sum of five skinfolds (correlation with DEXA % fat r=0.92), HOMA-IR, triglycerides, HDL cholesterol (HDL-C), HOMA-beta.
Results: Adiposity (sum of skinfolds) rose progressively and significantly between five and eight years (+18%, p<0.001), while HOMA-IR unexpectedly fell (-24%, p<0.05). Consistent with the reduction in HOMA-IR, HDL-C rose (+17%, p<0.001) and triglycerides fell (-8%, ns). Fasting glucose rose (+12% or 0.5 mmol, p<0.001), while HOMA-beta fell (-35%, p<0.001). The changes were similar in both genders.
Conclusion: Weight gain is normally associated with rising insulin resistance - but only when existing adipocytes are further filled and lose their triglyceride buffering capacity. Frayn's lipid flux hypothesis, on the other hand, envisages that if the increase in adiposity is due to new adipose cells, triglyceride buffering capacity will paradoxically increase, with a fall rather than rise in insulin resistance. The rise in adiposity, fall in HOMA-IR and rise in HDL-C reported here are consistent with the hypothesis. The rise in glucose, on the other hand, was unexpected, and may be related to an independent loss of beta cells, given the 35% fall in beta-cell function registered by HOMA-beta. These observations are novel, represent substantial metabolic remodelling in pre-pubertal children, and need explanation to establish whether they are physiological and programmed, or pathological and ultimately harmful. They should also be taken into account when interpreting the response to childhood interventions designed to reduce insulin resistance.
Support: Diabetes UK, Child Growth Foundation, Eli Lilly

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Risk factors of pre-diabetes in offspring of type 1 DM mothers
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Background and Aims: In a previous study we showed that young offspring of mothers with type1 diabetes(OMDM1) had a significantly higher prevalence of pre-diabetes compared to controls.The aim of this study was to investigate(a)any differences between the subgroup of OMDM1 who developed pre-diabetes compared to the normoglycemic OMDM1 subgroup;and b)any possible relationship between metabolic and hormonal characteristics of OMDM1 and the glycemic control indices of their mothers during pregnancy.
Materials and Methods: 31 prepubertal OMDM1 aged 5–10 years were examined. Their height, weight,BMI,body composition(using Futrex device),and blood pressure (BP)were measured.All children underwent an OGTT (1.75g gluc/Kg).Glucose, insulin and c-peptide were determined at 0,30&120 min after glucose load.Lipids, leptin,pro-insulin, as well as GAD65 and IA2 antibodies were measured.From pregnancy records the following data were also registered:duration of type 1 DM of mothers,age,pre-pregnancy BMI,initial HbA_{1c} during pregnancy,as well as HbA_{1c} through the three trimesters,and birthweight of newborns.Data regarding the occurrence of breastfeeding(≥ 1 month) were available for 23 of the 31 babies.

Results: The only child who was positive for the measured antibodies, developed type-1 diabetes. 8 of the 30 OMDM1 children(Group A) presented prediabetes (7 of them had impaired fasting glucose and 1 with impaired 2 h glucose),while 22 had normal OGTT(GroupB).GroupA children were heavier,(BMI: 21.3 ± 3.3 vs.18 ± 3.1 kg/m^2,p=0.026),had significantly higher fasting glucose (104 ± 10vs 90 ± 5 mg/dl,p<0.001), lower glucose to insulin ratio at 30 min(4.1 ± 2.4vs1.9 ± 0.4 p less than 0.05),and a tendency to higher systolic BP(103.7 ± 12 vs. 95.7 ± 10 mmHg, p=0.057). None of the Group A children were breast-fed (0/8),while more than half of GroupB children(10/16)were breast-fed(p=0.007).The absence of breast-feeding increased the occurrence of pre-diabetes tenfold [likelihood ratio positive(LR=10.3) p=0.001].Also an initial value of HbA_{1c} greater than 5.8%(the upper normal limit) during pregnancy increased the risk of prediabetes more than fourfold(LR=4.64,p=0.031).In stepwise logistic regression model, with child diagnosis as the dependent variable, breast-feeding and child BMI were independent predictors(predictive value=88.2%).The BMI of the children was significantly correlated with the mothers' second trimester HbA_{1c}(r=0.561, p=0.01)but not with the birthweight,breastfeeding,or the BMI of the mother. Finally a significant positive correlation was found between HbA_{1c} during pregnancy of all three trimesters and leptin(r=0.496 p=0.043,r=0.583 p=0.007and r=0.546 p=0.016 respectively)as well as fatweight(r=0.565 p=0.044,r=0.648 p=0.005 and r=0.564 p<0.012)of the children.
Conclusions: The intrauterine,hyperglycemic environment seems to play a significant role in the obesity of offspring of DM1 mothers, and in combination with the absence of breast-feeding seems to increase the risk of prediabetes in these children at an early age.

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Oxidative stress in offspring of mothers with poorly controlled gestational diabetes
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Background and Aims: Recent reports have identified maternal gestational diabetes mellitus (GDM) as a risk factor for the future development of endocrine and non-endocrine pathologies in offspring. But up to now the effect of unbalanced GDM on offspring metabolic features is still uncertain. In the present study we assessed the impact of pregnancy metabolic disturbances induced by poorly controlled maternal GDM on oxidative state balance in offspring.
Materials and Methods: Forty three offspring of mothers with poorly controlled GDM were consecutive recruited in different time after delivery. All GDM offspring (age: 19.3 ± 1.2 years; males/females: 23/20; BMI: 20.05 ± 0.57 kg/m^2) had fasting blood glucose and glucose tolerance test (OGTT) within normal values. Thirty three healthy volunteers (offspring of the women with normal carbohydrate metabolism during pregnancy) matched for age, sex and BMI served as controls. Serum lipid peroxidation products (conjugated dienes – CD, trienes, oxidienes, thiobarbituric acid reactive substances – TBARS) and antioxidative defence parameters (serum total antioxidant capacity - TAC, α-tocopherol levels) besides total cholesterol, triglyceride, HbA_{1c} and fructosamine were measured by spectrophotometry. Values reported as means±SEM.
Results: No differences in serum triglyceride, total cholesterol, HbA_{1c} and fructosamine between GDM offspring and controls were found (triglyceride: 0.71 ± 0.07 mmol/l vs 0.59 ± 0.08 mmol/l; total cholesterol: 4.76 ± 0.08 mmol/l vs 4.53 ± 0.11 mmol/l; HbA_{1c}: 4.65 ± 0.11% vs 5.4 ± 0.11%; fructosamine: 150.88 ± 5.15 mmol/l vs 148.35 ± 13.03 mmol/l). But compared to controls GDM offspring had strong significantly (p<0.001) higher values for serum lipid peroxidation products (CD: 0.57 ± 0.03 mmol/l vs 0.23 ± 0.05 mmol/l; trienes: 0.46 ± 0.02 mmol/l vs 0.29 ± 0.05 mmol/l; oxidienes: 0.27 ± 0.01 mmol/l vs 0.21 ± 0.03 mmol/l; TBARS: 1.71 ± 0.05 mmol/l vs 0.91 ± 0.06 mmol/l) as well as lower serum TAC (37.95 ± 1.18% vs 50.00 ± 2.43%) and α-tocopherol concentration (3.24 ± 0.12 μmol/l vs 8.19 ± 0.49 μmol/l).
Conclusion: Our study has demonstrated lipid peroxidation enhancement and antioxidative defence attenuation in offspring of mothers with uncontrolled GDM. Revealed oxidative stress in offspring against the normal lipid profile and nonimpaired glucose tolerance background may suggest a causal role of maternal metabolic disturbances due to GDM in pro/antioxidative system failure formation in GDM offspring (phenomenon in uteri „imprinting").

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Effect of age and gender on glucose production during intravenous glucose tolerance test
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Background and Aims: The role of age and gender on the ability of the liver to dynamically suppress and restore endogenous glucose production (EGP) after a glucose stimulus has not been studied in depth. The present study aimed to estimate EGP during a stable labelled intravenous glucose tolerance test (IVGTT) by applying deconvolution techniques to cold and tracer glucose data, and to assess whether EGP time course is affected by age and gender.
Materials and Methods: A 4-h (0–240 min), insulin-modified (20 mU/kgBW of insulin from 20 to 25 min) [6,6-$^2H_2$]glucose labelled IVGTT (330 mg/kgBW) was performed in 92 healthy subjects: 67 elderly (71 ± 1 years, mean±SE) (E) and 25 young (23 ± 1 years) (Y), 48 men (M) and 44 women (W). EGP was estimated by stochastic deconvolution, using the labelled two compartment minimal model to derive the impulse response.
Results: All groups were characterized by a similar, fast initial EGP suppression: starting from the basal value, the nadir (EGP_N -parameter values in the groups are shown in the table below-) and the time at which it was reached (t_N) were not significantly different. The recovery phase exhibits a similar pattern in M vs. W but not in E vs. Y, since the time at which EGP returns to basal (t_r) was lower in Y than in E (p=0.0003, Mann Whitney test). An overshoot of EGP was noted in the four groups. The maximum value of EGP (EGP_{max}) is similar in M vs. W, but higher in Y vs. E (p=0.0029). The time at which EGP_{max} is reached (t_{max}) is similar in the four groups. The global suppression (EGPS) resulting from suppression and recovery phases is lower in Y compared to E (p=0.0011): in Y the initial suppression phase is balanced by the overshoot in the recovery phase, while in E a net suppression results.

Group	EGP_N	t_N	t_r	EGP_{max}	t_{max}	EGPS
Men (M)	34.95 ± 5.21	54 ± 6	117 ± 9	134.67 ± 5.76	127 ± 12	10.01 ± 3.65
Women (W)	21.12 ± 4.98	49 ± 4	125 ± 9	139.42 ± 8.87	137 ± 14	14.56 ± 4.53
Elderly (E)	29.84 ± 4.23	55 ± 5	138 ± 8	126.93 ± 4.26	141 ± 12	17.84 ± 2.99
Young (Y)	24.29 ± 7.40	43 ± 4	82 ± 7 *	163.78 ± 14.10 *	108 ± 11	−2.96 ± 6.05 *
Mean±SE	28.33 ± 3.66	52 ± 4	121 ± 6	136.94 ± 5.18	132 ± 9	12.19 ± 2.88

Legend: EGP_N (percent of basal), nadir of EGP; t_N (min), occurrence of the nadir; t_r (min), time at which EGP returns to its basal value; EGP_{max} (percent of basal), maximum value reached by EGP; t_{max} (min), time at which EGP_{max} occurs; EGPS, area under EGP expressed as deviation from basal, from 0 to 240 min. (*) significantly different (p<0.05) with respect to E.

Conclusion: Age but not gender has an important effect on the ability of the liver to dynamically suppress and restore glucose production: elderly subjects suppress properly but lose the ability to recover promptly.

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Influence of gender in dysmetabolic hyperferritinemia syndrome. Metabolic and hepatic consequences
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Introduction: Previous studies have suggested that serum ferritin is one of the components of the insulin resistance syndrome in Caucasians. Sheu WH., in Taiwan, observed that a relationship between serum ferritin levels and insulin resistance exists in women but not in men. In other studies, iron stores were positively associated with risk factors of cardiovascular disease (CVD) only among non-Hispanic black and Mexican American women after adjustment for confounding variables.
Aims:
1- To define the anthropometric, metabolic and haemodynamic characteristics of patients with hyperferritinemia.
2- To evaluate ferritin levels in patients with/without metabolic syndrome.

Methods: N=113 patients, aged 39–81 (59,34 ± 0,86). 55/58 male/female ratio. 64 with metabolic syndrome (SM) defined by ATP-III criteria. Patients with genetic haemochromatosis (GH), associated with mutations of the HFE gene (Cys282Tyr and His63Asp), were excluded. Waist (cm) and waist/hip ratio. Glucose, HDL, LDL, uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltranspeptidase (GGT) and alkaline phosphatase, assessed by Hitachi autoanalyzer.
HbA1c (%): HPLC. Serum ferritin (ng/ml): ADVIA Centaur Bayer. Ambulatory Blood Pressure Monitoring: SpaceLabs 90207. Statistical analysis: Student's T test. Mann-Whitney's U test. Kolmogorov-Smirnov's test. Chi-Squared test.
Results:
1 – Patients with MS have higher levels of ferritin (p=0,019) compared to those without; with similar ages in both groups (60,73 ± 1,07 vs 57,51 ± 1,36; non-statistically significant). Adjusting by gender, only women maintain these differences (96,09 ± 14,66 vs 60,39 ± 16,01; p=0,004).
2 – Hyperferritinemia (HF) adjusted by gender: Men: ferritin ≥200; cut-off above the 69th percentile. Women: ferritin ≥100; cut-off above the 72nd percentile. HF was diagnosed in N=35 patients (31%), 19 males (54,3%), 16 females (45,7%).
3 – The prevalence of HF among patients with MS was of 37,5%, whereas among patients without MS it was of 22,4%, p=0,05. Adjusting by gender, the prevalence of HF in males with MS was 35,3%, versus 33,3% in those without MS (non-significant), whereas in females it was 40% vs. 14,3% (p= 0,028), respectively.
4 – In males, with similar ages between groups (54,37 ± 1,80 vs 58,08 ± 1,43), those with HF had higher waist circumference values (99,79 ± 1,90 vs 93,04 ± 1,58; p=0,007) than those without. This was the only finding belonging to the MS. No significant differences were found regarding hepatic serum enzymes.
5 – In females, at similar ages, those with HF had higher levels of triglycerides (p=0,034) but lower levels of HDL (51,06 ± 2,13 vs 60,54 ± 2,48; p=0,036). There were significant differences in hepatic enzyme values: ALT (p<0,001) and GGT (p=0,010).
6 – Hyperferritinemia is not associated with high blood pressure in males or females.
Conclusion:
1 – Patients with MS have higher levels of serum ferritin.
2 – There is a sexual dimorphism in its relation with the MS which merits further investigation.
3 – The consequences of iron overload are anthropometric in men but metabolic and hepatic in women. No differences were found in blood pressure values between both groups.
4 – Venesection therapy or phlebotomy could be effective in controlling iron overload and its metabolic and hepatic consequences.

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Effect of dehydroepiandrosterone (DHEA) on insulin resistance and DHEA-sulfate concentration in male adults of Japanese longevity district

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Background and Aims: To clarify the effect of dehydroepiandrosterone (DHEA) on insulin-induced glucose uptake, activation of phosphatidylinositol (PI) 3-kinase-atypical PKC and 5'AMP-activated protein kinase (AMPK) which is known as a second messenger of adiponectin, and the association of DHEA with longevity of male adults in Japan, we investigate whether DHEA affects AMPK activation with PI 3-kinase-atypical PKC activation in rat adipocyte and measured DHEA-S concentration in male adults of Japanese longevity ditrict.
Materials and Methods: We have investigated the effects of DHEA on insulin-induced glucose uptake, expression of peroxisome proliferator-activated receptor gamma (PPARr) of adipocytes in Otsuka Log-Evans Tokushima fatty rat (OLETF), type 2 diabetes animal models. We measured serum DHEA-sulfate (DHEA-S) concentration, fasting plasma glucose (FPG), 2 hr-plasma glucose after 75g oral glucose loading (2 hrPG), fasting insulin (FIRI) and HbA1c, adiponectin of 34 male adults in Kokufu-cho, most longevity district (mean life span in male: 80.4 yr-old) and those of 559 male adults in Kasamatsu-cho (mean life span: 77.6 yr-old) as control.
Results: DHEA reduced epididymal and perirenal adipose tissue in association with decreased plasma leptin levels in OLETF. Adipose tissue from OLETF showed increased expression of PPARr protein, which was prevented by DHEA treatment. DHEA significantly reduced mRNA levels of PPARr, adipocytes lipid-binding protein and sterol regulatory element-binding protein in primary cultured adipocytes. However, DHEA activated PI 3-kinase-PKCzeta signaling without AMPK activation. On the other hand, there was a significant difference of mean age between in kokufu-cho (72 ± 7 yrs) and Kasamatsu-cho (51 ± 8 yrs) (P < 0.0001). FIRI level in Kasamatsu-cho (7.4 ± 3.3 mU/ml) was significantly higher than in Kokufu-cho (5.3 ± 4.0 mU/ml) (P < 0.005). There were no significant differences of BMI, body fat compsition, diastolic pressure, total cholesterol, LDL-cholesterol, FPG and HbA1c, but QUICKI (quantative insulin sensitivity check index) is significantly higher and HOMA-R was significantly lower in Kokufu-cho than those in Kasamatsu-cho (P < 0.001). Serum DHEA-S concentrations of male adults in Kokufu-cho, longevity district of Japan was significantly higher than in Kasamatsu-cho as control, if adjusted for age, BMI, blood glucose (p < 0.0001). However, no correlations between DHEA-S concentration and FPG, HbA1c, insulin sensitivity indices and adiponectin were found. Negative correlation of DHEA-S with age was expectedly found (P < 0.0001) in total 593 male adults. Serum adiponectin levels were negatively correlated with BMI (P < 0.001) and body fat compsition (P < 0.01), respectively, in 34 male adults of Kokufu-cho.
Conclusion: DHEA treatment provokes glucose uptake through a PI 3-kinase-PKCzeta pathway without AMPK activation and downregulates adiposity through the reduction of PPARr in rat adipocytes. Finally, DHEA may contribute to longevity coincident without serum adiponectin level in elderly aged male adults.

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Fasting glycaemia, serum insulin and C-Peptide, in healthy centenarian subjects

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Background and Aims: There is still big controversy on concerning the presence of carbohydrate metabolism impairment in advanced age. Insulin in serum has been a key parameter for carbohydrate metabolism studies. High accuracy and precision has been reached from data obtained at the beginning by the rat - diaphragm method to the to-day available highly specific and sensitive non-isotopic immunoassays used for blood insulin measurements. We used one of these recent methodologies to investigate the status of carbohydrate metabolism in centenarians. Moreover we evaluated connecting peptide (C-Peptide) by the use of non isotopic luminescence assay.
Materials and Methods: We measured serum fasting glycaemia, insulinaemia and C-Peptide in tree subject groups: i) 28 normal adult subjects, of both gender, (age range 18–62 yr); ii) 25 normal elderly subjects, of both gender (age range 66–91 yr); iii) 26 healthy centenarians (8 men and 18 women (age range 100–105 years). All tree groups were selected by the criteria of the Eurage Senieur Protocol. All subjects gave a full social and medical history and underwent physical examination. In particular, they were mentally competent to give oral and written informed consent. We determined serum glycaemia values by glucoso-oxidase method, serum insulin and serum C-Peptide levels by the electrochemiluminescence immunoassay ECLIA method (Roche Elecsys 1010/2010 and modular analytics E170 (Elecsys module) immunoassay analyzers.
Results: Fasting glycaemia levels (mg/dl±SE) were respectively: i)95 ± 8; ii)94 ± 9; iii) 96 ± 12. Insulinaemia levels (mU/±SE) were: i) 13.03 ± 3.84; ii) 9.90 ± 3.26; iii) 13.50 ± 2.89. C-Peptide values (ng/ml±SE) were: i) 0.82 ± 0.03; ii) 0.78 ± 0.08; iii) 0.38 ± 0.06. Fasting glycaemia and insulinaemia appeared unmodified in the tree subjects groups under investigation, whereas C-Peptide showed a significant reduction (P<0.01) in centenarians.
Conclusions: In this study serum glucose levels and insulinaemia, in centenarians, appeared unchanged in comparison to adult and elderly normal subjects. C-Peptide was significantly reduced in centenarians in comparison to normal adult and elderly subjects, although still in the low level of normal range. The single significant reduction (P<0.01) in plasma C-Peptide in centenarians does not seem to be relevant with an impaired glucose tolerance, but most probably with disturbances of C-Peptide clearance in the liver.
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Free fatty acids concentration and composition is associated with family history of diabetes mellitus type 2 in healthy Czech subjects

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Background and Aims: The circulating free fatty acids (FFAs) are key mediator of insulin sensitivity. Elevated FFAs contribute to the hyperinsulinemia and chronic exposure to elevated FFA levels can be damaging to the B-cell function. Numerous studies have demonstrated that saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA) do not influence glucose and lipid metabolism in the same way. Dietary PUFA improve the anomalies in glucose and lipid metabolism associated with fat ingestion. MUFA intake has also been shown to be beneficial. In contrast, deleterious effect of SFA was found. Our aim was to assess and compare the FFA plasmatic concentration and composition in healthy adults with and without family history (FH) of DM2 and to analyse the association of the FFA concentration and composition with biochemical parameters related to glucose and lipid metabolism, markers of insulin sensitivity (1/HOMA R, Matsuda index), B-cell function (HOMA F, Insulinogenic index), disposition index, and body composition.
Materials and Methods: We studied FFA concentration and composition (proportion of PUFA, MUFA, and SFA) in 324 nondiabetic subjects (M/F 99/225; age 32 ± 11years; BMI=23.9 ± 4.0 kg/m^2). OGTT and ivITT were performed. The FFA basal and oGTT-stimulated concentrations were evaluated by Wako NEFA C ACS-ACOD method and FFA composition was assessed by GC method. For the statistical analysis, NCSS 2004 software was used.
Results: The comparison of basal FFA concentration between the subjects with and without FH of DM2 revealed higher FFA levels in DM2 offspring (p=0,009). Also oGTT-stimulated levels of FFA were slightly higher in DM2 offspring (p=0,05). In terms of FFA composition, subjects with FH of DM2 displayed significantly lower PUFA proportion (p=0,0005) as well as lower PUFA/SFA ratio (p=0,009). These observations correspond with higher proportion of DM2 offspring in a group of subjects above the upper quartile of basal FFA conc. (Chi^2=4,4; p=0,04) and also in a group below the lower quartile of PUFA/SFA ratio (Chi^2=10,0; p=0,002). Regarding the basal FFA concentration and its relation to biochemical and anthropometric data, significant association between low basal FFA conc. (below the lower quartile) and beneficial lipid profile (lower TG and total as well as LDL cholesterol levels) was observed. High basal FFA levels were significantly associated with higher oGTT-stimulated glucose levels (p=0.000) both in subjects with and without FH of DM2. Also oGTT-derived indices of insulin sensitivity and B-cell function were significantly worse in subjects with high basal FFA levels. Furthermore, slightly higher BMI and waist circumference were found among subjects with FFA conc. above the upper quartile. However, according to our observation, FFA composition does not associate with insulin sensitivity, B-cell function or body composition.
Conclusion: The present study demonstrates that positive FH of DM2 is associated with higher basal FFA levels and also with lower PUFA/SFA ratio. Regardless FH of DM2, subjects with high basal FFA concentration display significantly higher oGTT-stimulated glucose levels and disfavourable values concerning indices of insulin sensitivity and B-cell function.
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PS 47
Nitric oxide and blood flow

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Involvement of nitric oxide in the *in vivo* increased pancreatic B-cell responsiveness to glucose in 48h lipid-infused rats

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Background and aims: Dysregulation of fatty acid (FA) metabolism is a common feature of metabolic disorders, such as obesity complicated or not with type 2 diabetes. In the prediabetic phase pancreatic B-cell hypersecretes insulin despite normal blood glucose level. FA may modulate glucose-induced insulin secretion (GIIS) by acting on B-cells both indirectly and directly. We previously showed that lipid infusion in normal rat induced a decrease in sympathetic nervous system activity thus leading to exaggerated GIIS. In the present study, whypothesized that L-arginine-derived nitric oxide (NO) may also be a mediator of FA effect on increased GIIS. Indeed, islet NO system is an important regulatory factor in insulin and glucagon secretion. Furthermore, endothelial NO synthase (NOS) gene expression is up-regulated by FA, such as linoleate.
Materials and methods: Two month old Wistar rats were intravenously infused with Intralipid (ie IL rats) or saline during 48 h. For intravenous infusion, a catheter was inserted into the right atrium via the jugular vein. At the end of infusion, GIIS was measured in response to a single ip glucose injection (1 g/kg bw). To test the role of NO, GIIS experiments were also performed in presence of NG-monomethyl-L-arginine (L-NMMA, a NOS inhibitor) or saline. To that end, a single dose of L-NMMA (5 or 20 mg/kg bw) was ip injected 5 min prior to glucose load. In another serie of experiment, pancreases were removed and islets were isolated to measure both NOS activity and gene expression of inducible, endothelial and neuronal NOS isoforms using quantitative RT-PCR method.
Results: During lipid-infusion period, both plasma insulin and glucose remained unchanged in IL compared to controls. In contrast, there was an exaggerated GIIS in IL rats. Consequently, the insulinogenic index was about 2.5-fold greater in IL than in control rats. In presence of L-NMMA, exaggerated GIIS was totaly abolished. Total NOS activity was increased by about 3 times in IL rats compared to controls. In contrast, NOS isoform expression remained unchanged.
Conclusion: We conclude that in vivo effect of FA on GIIS is partly mediated by NO. Dysregulation of NO pathway could be an early mechanism which could lead to further alteration of pancreatic B cell function.

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Portal delivery of a nitric oxide synthase inhibitor enhances net hepatic glucose uptake in the conscious dog

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Background and Aims: Changes in hepatic nitric oxide (NO) are proposed to regulate muscle insulin sensitivity by bringing about the hepatic release of a humoral factor termed the hepatic insulin sensitizing substance, or HISS. However, previous studies evaluating HISS have been conducted in the presence of euglycemia, when the liver's role in glucose disposal is minimal. The liver and muscle usually play reciprocal roles in glucose disposal. Therefore we hypothesized that reduction in hepatic NO levels would enhance net hepatic glucose uptake (NHGU) while reducing nonhepatic glucose disposal under hyperglycemic, hyperinsulinemic conditions.
Materials and Methods: Studies were conducted on 7 chronically catheterized (sampling catheters in the femoral artery, hepatic portal vein, and hepatic vein; infusion catheters in a jejunal and splenic vein) conscious 42 h-fasted dogs of either sex. Arteriovenous difference and tracer methods were used.Studies consisted of a 90 min tracer ([3-^{3}H]glucose) equilibration period, a 30 min basal period, and two 90-min experimental periods (P1 and P2). During P1 and P2, dogs received somatostatin via peripheral vein; insulin and glucagon via portal vein at 4-fold basal and basal rates, respectively; and peripheral glucose infusion to increase the hepatic glucose load 2-fold basal. During P2, the NO synthase inhibitor L-NAME was also infused intraportally at 0.3 mg/kg·min.
Results: The arterial plasma insulin concentrations were ~3.5-fold basal during P1 and P2 (basal, 34 ± 9; P1, 118 ± 19; P2,119 ± 19 pM), and arterial

plasma glucagon averaged 42 ± 4 ng/l in all periods. During L-NAME infusion, hepatic blood flow decreased from 27.3 ± 1.5 to 17.4 ± 1.0 ml/kg · min, mean arterial pressure increased from 115 ± 6 to 130 ± 10 mmHg, and heart rate fell from 83 ± 11 to 46 ± 3 bpm (P<0.05 for all changes). Plasma cortisol did not change significantly. The following results are detailed in Table 1. A significantly higher glucose infusion rate was required in P2 to maintain the hepatic glucose load equivalent in P1 and P2. Net hepatic glucose uptake and fractional extraction increased significantly during P2. Nonhepatic glucose clearance tended to increase between P1 and P2 (P=0.06). The rate of whole body glucose disappearance (R_d) increased significantly during P2.

Conclusion: Inhibition of hepatic nitric oxide synthase (NOS) stimulates net hepatic glucose uptake and tends to increase nonhepatic glucose disposal under hyperglycemic, hyperinsulinemic conditions. These effects significantly enhance whole body glucose disposal. Pharmacologic modulation of NOS might reduce postprandial glucose excursions.

Table 1. Glucose infusion rate, hepatic glucose load, and glucose disposal

	P1	P2
Glucose infusion rate (μmol/kg · min)	28.0 ± 3.5	58.8 ± 7.9*
Hepatic glucose load (μmol/kg · min)	210 ± 14	189 ± 11
Net hepatic glucose uptake (μmol/kg · min)	11.3 ± 2.5	20.1 ± 1.6*
Net hepatic glucose fractional extraction	0.055 ± 0.012	0.108 ± 0.009*
Nonhepatic glucose clearance (ml/kg · min)	2.7 ± 0.6	3.8 ± 0.7
Glucose R_d (μmol/kg · min)	37.8 ± 4.1	55.8 ± 9.8

*P < 0.01 vs P1; P < 0.05 vs P1

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Negative regulation of Akt phosphorylation by JNK in insulin-signaling pathways related to high glucose inhibited nitric oxide production in human vascular endothelial cells

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Background and Aims: Insulin has vasodilator actions and has been demonstrated to simulate nitric oxide(NO)production in endothelial cells. It has been proposed Akt contribute to this process, but other aspects of insulin-signaling pathways have not been elucidated. Recent reports has suggested that high glucose inhibits insulin-stimulated NO production in endothelial cells. But the molecular mechanisms are not well understood. In this study, we explore the role of c-Jun NH_2-terminal kinase (JNK), insulin receptor substrate-1(IRS-1), and Akt phosphorylation and their cosstalk in this process.
Materials and Methods: human umbilical vein endothelial cells(HUVECs) were exposed to high glucose(30 mM), and 5 mM glucose supplemented-with 25 mM mannitol as hyperosmolarity for 24 h. For control experiments, parallecultures were exposed to normal glucose(5 mM). Cells were then treated with insulin for 20 min. In some experiments, SP600125(10um) and wortmannin (50 nM) were added 20 min before treatment with insulin. Production of NO was assessed using the NO-specific fluorescent dye 4,5-diaminofluorescein diacetate (DAF-2 DA). Phosphorylation status of JNK, IRS-1, and Akt was determined by immunoblotting with phospho-specific antibodies to JNK(Thr^{183}/Tyr^{185}), IRS-1($Ser^{307/312}$), and Akt (Ser^{473}) Antibody.
Results: Incubation of HUVECs at high glucose vs normal glucose for 24 h resulted in a significient decrease in insulin-stimulated NO production (0.45fold vs 1.00fold, p< 0.001), whereas osmotic control had no effect on it. Prior incubation of JNK inhibitor could abolished the effect of high glucose(0.90fold vs 0.45fold, p<0.01). Treatment with high glucose increased phosphorylation of JNK(1.82fold vs 1.00fold, p< 0.01) and Ser^{312} IRS-1(1.86 fold vs 1.00fold, p< 0.01), and decreased phosphorylaiton of Akt(0.63fold vs 1.00fold, p<0.05). JNK inhibitor SP600125 reduced Ser^{312} phosphorylation of IRS-1(0.67fold vs1.86fold, p<0.01), and enhanced phosphorylation of Akt(0.93fold vs 0.63fold, P<0.05). PI3K inhibitor wortmannin could aggravate the effect of high glucose(0.31fold vs 1.00fold, p<0.05). Wortmannin decreased the phosphorylation of Akt(0.50 fold vs 0.93fold, p< 0.01), but it had no effect on JNK and IRS-1.
Conclusion: Our results show that high glucose inhibits insulin-stimulated NO production and up-regulated some aspects of insulin signaling, inculding the JNK signaling pathway. And negative regulation of Akt by JNK through phosphorylating Ser307 in IRS-1 leads the less insulin-simulated NO production.

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Effects of myocardial overexpression of endothelial nitric oxide synthase on ventricular dysfunction and ischemia/reperfusion injury in diabetic mice

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Background and aims: Diabetes is associated with a number of cardiovascular complications. Recent studies have shown that NO is implicated in diabetic myocardial dysfunction, but whether NO generation is beneficial or deleterious in the development of diabetes-induced myocardial diseases is still under debate. We therefore used a transgenic mouse model of myocyte-specific endothelial nitric oxide synthase (eNOS) overexpression to investigate the role of NO in the development of diabetic myocardial dysfunction.
Methods: Diabetes was induced in transgenic mice that overexpress eNOS exclusively in cardiac myocyte (TG) and their corresponding non-transgenic littermates (WT) by a single intraperitoneal injection of streptozotocin (200 mg/kg). Untreated age-matched WT and TG animals served as controls. Four weeks after induction of diabetes, hearts were retrogradely perfused at constant flow (2 mL/min) and subjected to no-flow ischaemia followed by reperfusion. Left ventricular developed pressure (LVDevP; balloon method), left ventricular end-diastolic pressure (LVEDP), maximum rate of rise and fall of left ventricular pressure, heart rate and coronary perfusion pressure were recorded and compared between experimental groups (n=5 [control] and 10 [diabetic], respectively).
Results: Both at baseline and following beta-adrenergic stimulation with norepinephrine (3–3000 nM), left ventricular performance was worsened in diabetic TG compared to diabetic WT hearts perfused under normoxic conditions (P<.05). In contrast to normoxia, the transgene significantly protected diabetic hearts during ischemia and reperfusion resulting in reduced ischemic contracture, a lower rise in diastolic pressure and improved recovery of reperfusion contractile function (relative to respective baseline). All these effects were significantly attenuated by blocking NO synthases with Nω-nitro-L-arginine methyl ester administered in vivo at 50 mg/kg per day for 2 days. Bradykinin (0.01 mM), via generation of endogenous NO, more strongly reduced myocardial O_2 consumption in diabetic TG than diabetic WT hearts perfused in normoxia (P<.05), whereas there was no difference after ischemia/reperfusion.
Conclusion: These data suggest that cardiac myocyte-specific overexpression of eNOS contributes to depressed basal cardiovascular contractile function in diabetic hearts and to contractile hyporesponsivness to beta-adrenergic stimulation, but efficiently protects the heart during ischemia/reperfusion stress, probably in part through an O_2 sparing effect.
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Modulation of mitochondrial Nitric Oxide Synthase by insulin in rat skeletal muscle

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Background and Aim: Postranslational modifications, as N-terminal acylation and Serin 1412 phosphorylation, are associated to Nitric Oxide Synthase traslocation to mitochondria in different tissues (mtNOS); mtNOS synthesizes nitric oxide (NO) vectorially directed to the mitochondrial matrix. It is noteworthy that NO steady-state concentration modulates electron transfer, O2 uptake, and reactive oxygen species yield. Serin 1412 is an AKT-dependent domain and considering that insulin activates this Kinase *via* PI3K, we analyzed insulin effects on rat skeletal muscle mtNOS.
Materials and Methods: Wistar rats (200–250g) were subcutaneously inoculated with 0.1U/Kg insulin glargine (I) or ClNa 0.9% and afterwards extensoris digitorum and soleus muscles were excised at 3 and 48 h. The isolation and purification of mitochondria was made by differential centrifugation at 3 and 48 h and in isolated muscles incubated with insulin and LY294002 (PI3K specific inhibitor). The mtNOS activity was determined by conversion of L-[H3] arginine to L-[H3] citrulline and the expression of mtNOS, p-AKT, and AKT1 and 2 were determined by Western Blot. We measured the p-AKT activity and mtNOS phosphorylation by immunoprecipitation. The mitochondrial p-AKT and NO were also detected by flow cytometry. Mitochondrial O2 uptake was determined polarographically with a Clark-type electrode placed in chamber at 30 °C in reaction medium saturated with room air, with 0,3 mg protein/ml and 6 mM malate-glutamate as substrate, in the presence or absence of phosphate acceptor (ADP). Data were expressed as mean±

S.E.M and analyzed by ANOVA and Dunnett test. Statistical significance was accepted at p<.05
Results: 1) Insulin increased the mtNOS activity at 48 h (in pmoles/min.mg prot: C: 30 ± 0.7; 3 h: 35 ± 2.6, 48 h: 145.8 ± 3.0, p<.05) without changes of protein content. Accordingly, this activity was inhibited by LY294002 in muscle homogenates (C: 27.5 ± 2.9; I: 100.4 ± 7.5; LY: 49.5 ± 5.26; LY + I : 20.2 ± 3.09) 2) At 48 h, mitochondrial NO yield increased and O2 uptake decreased, respect to controls or to 3 h samples (in ngat O/ min.mg prot: C: 53 ± 11; 3 h: 88 ± 5; 48 h: 33 ± 6). 3) At 3 h, p-AKT increased in cytosol and traslocated to mitochondria, where expression resulted increased by 40%, and turned back to control levels at 48 h 4) in accord, at 3 h p-AKT activity was higher in cytosol and mitochondria respect to controls and to 48 h samples 5) At 3 h, AKT2 protein level was increased by 59% in cytosol and 21% in mitochondria, while AKT 1 expression was unaltered 6) mtNOS was phosphorylated by p-AKT.
Conclusions: p-AKT2 activated *via* PI3K-Insulin and translocated to skeletal muscle mitochondria increases the mtNOS activity by phosphorylation. Likewise, this effect reduces O2 and substrate uptake by mitochondria and favors insulin-dependent biosynthetic and anaplerotic pathways, as glucogenosynthesis.

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Implication of neuronal NO synthase and its protein inhibitor PIN in glucose uptake by L6 myocytes
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Background and Aims: We previously demonstrated that rat pancreatic β-cells express neuronal NO synthase (nNOS) and its endogenous inhibitor PIN, and which both play a role in the control of insulin response to glucose. As skeletal muscle is known to express a splice variant of nNOS, nNOSμ and as PIN has been identified in rat ventilary muscles, we then questioned whether nNOS and PIN could modulate peripheral insulin action, especially in skeletal muscle cells. To study nNOS and PIN function in myocytes, we used L6 cell line and first analysed the expression profile of the two proteins during the differentiation of the cells into myocytes over a period of three weeks. We then measured glucose uptake in the presence of a pharmacological inhibitor of nNOS, Nω-nitro-L-arginine methyl ester (L-NAME), an NO donor, sodium nitroprusside (SNP), and after overexpression of PIN in the myocytes.
Materials and Methods: nNOS and PIN expression was analysed by quantitative RT-PCR at different differentiation stages of L6 cells (myoblats and myocytes). Glucose uptake was measured during a 5 min incubation with 2-[^{3}H]-2-deoxy-D-glucose after a one-hour preincubation period with or without 100 nM insulin and the tested compounds.
Results: Both nNOS and PIN were found to be expressed in myoblasts. PIN mRNA increased strongly after the first week of differentiation and remained stable during further differentiation to myocytes (second and third week). Concerning nNOS, mRNA expression steadily increased throughout the differentiation period tested. At the functional level, increasing concentrations of L-NAME (5 and 10 mM) produced a dose-dependant decrease in glucose uptake in the absence (–33% for 10 mM, P<0.01) or in the presence of insulin (–19% for 5 mM, P<0.05; -46%, P<0.001). To confirm that the effects of L-NAME were due to a decreased NO production, we tested the NO donor SNP (30, 300 μM and 3 mM), that produced a significant stimulation of glucose uptake at the 3 mM concentration in the presence of insulin (+ 39%, P<0.01). As concerns PIN, its overexpression surprisingly induced an increase in glucose uptake independently of the presence of insulin (+46% without, P<0.001; +17% with insulin, P<0.05).
Conclusion: nNOS and PIN, expressed in L6 myocytes, are both positive modulators of glucose uptake in L6 myocytes. If nNOS effect is probably related to NO production, the unexpected effect of PIN, also known as a component of the cytoskeleton, might be related to an improvement of GLUT4 translocation to the plasma membrane.

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Endothelin-1 vascular and resultant metabolic actions in perfused rat hindlimb are opposed by insulin
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Background and Aims: Insulin stimulates the release of both ET-1 and nitric oxide (NO) from the endothelium, and it has been proposed in various studies that the hemodynamic effects of insulin are a balance between the vasodilator (NO) and the vasoconstrictor (ET-1) effects
Endothelial dysfunction is associated with an increase in the formation and release of ET-1; elevated levels have been detected in type 2 diabetics, obese patients and in hyperinsulinemic states in hypertensive individuals. In hypertensives ET-1 has been shown to impair the vasodilator response to exercise. Raised ET-1 levels have been found to lower whole body insulin-mediated glucose uptake as well as peripheral glucose uptake, including leg glucose uptake. It has been proposed that the insulin resistance observed in cases with elevated ET-1 is due to vasoconstriction causing reduced skeletal muscle perfusion by ET-1, and therefore reduced delivery of insulin and glucose to skeletal muscle.
Our aim was to determine the dose-dependent effects of ET-1 on hemodynamics and metabolism, of the constant-flow pump-perfused rat hindlimb and to determine whether interaction occurs between endothelin and insulin.
Materials and Methods: Initially we attempted to distinguish the vascular from the metabolic effects of ET-1 in the constant-flow pump-perfused rat hindlimb by using various doses of ET-1 and measuring changes in perfusion pressure (PP), oxygen consumption (VO_2), glucose uptake (GU), and lactate release (LR). Nitroprusside (SNP) was used to block vasoconstriction and to thus assess the relationship between vascular and metabolic effects. Insulin (15nM) was included in later experiments to determine the interaction between insulin and ET-1 on the above parameters.
Results: ET-1 caused a dose-dependent increase in perfusion pressure. Effects on oxygen consumption were biphasic, with low doses increasing oxygen consumption (by approx. 7 $\mu mol \cdot g^{-1} \cdot h^{-1}$ at 1nM, $P<0.05$ vs control), and higher doses leading to a net inhibition (approx -7 $\mu mol \cdot g^{-1} \cdot h^{-1}$ at 10nM, $P<0.05$ vs control). Glucose uptake and lactate release was increased at lower doses (ET-1 ≥1nM, 30 $\mu mol \cdot g^{-1} \cdot h^{-1}$ and 65 $\mu mol \cdot g^{-1} \cdot h^{-1}$ respectively, $P<0.05$ vs control), but this effect was lost at higher doses (≤10nM ET-1). SNP (50 μM) fully blocked the increase in pressure and metabolism due to low dose ET-1 and partly blocked both pressure and metabolic responses by the high dose. ET-1 vasodilatory activity was minimal at high or low dose. In the presence of 15 nM insulin ET-1 vasoconstrictor activity to alter metabolism was markedly inhibited. Low dose ET-1 mediated increase in pressure, oxygen and lactate output were completely blocked by insulin. High dose effects of ET-1 to further increase pressure were partly blocked and the effects to decrease metabolism were converted to outcomes resembling low dose ET-1. The effect of low dose ET-1 to increase glucose uptake was blunted by insulin such that the two were not additive, and the sum of high dose ET-1 plus insulin was indistinguishable from low dose ET-1 plus insulin.
Conclusion: Overall, these results show that ET-1 has a biphasic dose-dependent vasoconstrictor effect on hindlimb blood vessels, able to modulate flow to cause both the stimulation and inhibition of metabolism. These effects of ET-1 are strongly opposed by insulin, which is able to vasodilate against both low and high doses of ET-1. We believe these interactions are important to our understanding insulin's action to control blood flow distribution in muscle.
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Vasodilatation does not increase capillary permeability for glucose or transport of insulin to muscle tissue in obese subjects
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Background and Aims: In addition to cellular insulin resistance obese subjects have a delay in insulin action and delivery of insulin to muscle interstitial fluid after glucose/insulin stimulation. Also insulin-stimulated capillary recruitment is diminished in insulin resistant states. This study was designed to investigate whether acute vasodilatation could increase delivery of insulin, muscle glucose uptake and capillary recruitment in skeletal muscle in obese subjects during an oral glucose load.
Materials and Methods: 9 obese individuals (age 48 ± 3yrs, BMI 31 ± 3 kg/m^2, waist circumference 106 ± 8 cm) were investigated during an oral glucose tolerance test (OGTT). In one arm intraarterial Metacholin (2 μg/min) was infused for 60 min during the beginning of the OGTT. The contralateral arm was control. In both arms glucose and insulin measurements in muscle interstitial fluid (with the microdialysis technique) were combined with forearm A-V catheterization and blood flow measurements (with venous occlusion plethysmography). Capillary recruitment was estimated by calculations of permeability surface area product (PS) for glucose.
Results: Blood flow increased significantly in the vasodilated arm but not in the control arm. Muscle glucose uptake and permeability surface area

product (PS) increased similarly in both arms during the oral glucose load with no difference between vasodilated and control arm (see table). Interstitial muscle insulin increased similarly in both arms during the OGTT
Conclusion: PS, but not blood flow, was increased by OGTT. Blood flow, but not PS, was further increased by metacholin.
Transcapillary delivery of insulin to muscle interstitial fluid as well as muscle glucose uptake and PS for glucose was unchanged by acute vasodilatation with metacholin during an oral glucose load.

Blood flow, PS glucose and glucose uptake during OGTT

Time	0 min	60 min	p-value
Bloodflow (mL/min/100g)			
Vasodilated arm	2.5 ± 0.8	7.2 ± 1.8	P<0.001
Control arm	2.2 ± 0.4	2.0 ± 0.3	ns
PS glucose (mL/min/100g)			
Vasodilated arm	0.1 ± 0.2	0.7 ± 0.4	P<0.001
Control arm	0.1 ± 0.2	0.9 ± 0.4	P<0.001
Glucose uptake (µmol/min/100g)			
Vasodilated arm	0.1 ± 0.5	4.5 ± 1.9	P<0.001
Control arm	0.1 ± 0.2	4.6 ± 1.1	P<0.001

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Increased skeletal muscle blood flow in patients with longstanding type 1 diabetes and no microvascular complications
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Background and Aims: Microvascular complications, including retinopathy, nephropathy and neuropathy are found in the majority of the patients with longstanding type 1 diabetes mellitus. Although the exact pathophysiology of microvascular complications is unclear, an increased capillary blood flow and capillary pressure, resulting in endothelial injury and subsequent microvascular sclerosis is viewed as a key phenomenon. According to this "haemodynamic hypothesis", the increased tissue perfusion predisposes to microangiopathy in diabetic patients. In a previous study, we found that patients with uncomplicated type 1 diabetes and a duration of disease of less than ten years had increased skeletal muscle blood flow compared to age- weight- and sex-matched nondiabetic subjects. In the present study, we investigated whether patients with *uncomplicated* type 1 diabetes mellitus (DM) and a diabetes duration of more than twenty years had elevated tissue perfusion and whether this was due to abnormal functioning of the sympathetic nervous system, being one of the main regulators of vascular tone.
Materials and Methods: In 6 DM patients (age 43,4 ± 0.5 years; DM duration 25.3 ± 2.6 yr.; HbA1c 8.5 ± 0.7%), who had no evidence of microvascular complications (no retinopathy, no neuropathy and no nephropathy) and 6 age- and sex matched healthy volunteers (Control), we measured hemodynamic parameters including forearm blood flow (FBF; plethysmography) and sympathetic nerve activity and function by the combination of arterial plasma sampling (catecholamine levels), microneurography and power spectral analysis of blood pressure and heart rate.
Results: At baseline, FBF was clearly increased in DM (FBF 4.8 ± 1.2 and 2.2 ± 0.3 mL · dL^{-1} · min^{-1}, DM and Control respectively; p<0.05) and forearm vascular resistance (FVR) was decreased (FVR 25 ± 6 and 43 ± 3 AU, DM and Control respectively; p<0.05). Heart rate was higher in DM (HR 77 ± 10 and 57 ± 2 beats/min, DM and Control respectively; p<0.05). Systolic and diastolic blood pressures were similar in both groups.
Arterial catecholamine concentrations, muscle sympathetic nerve activity and heart rate and blood pressure variability were not different between DM and Control.
Conclusion: In patients with type 1 diabetes, without signs of microvascular complications and a diabetes duration of more than twenty years, skeletal muscle blood flow is clearly increased. This increased blood flow can not be attributed to disturbances in sympathetic nervous system activity. The results of this study challenge the view that increased blood flow plays a causative role in the pathophysiology of microvascular complications.
Support: Dutch Diabetes Foundation

PS 48
Fatty acids and lipids

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The regulation of fatty acid release from adipose tissue in vitro
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Background and Aims: The elevated non-esterified fatty acid levels (NEFA) in serum are considered to be one of the main causes leading to the development of the insulin resistance. The aim of our study was to determine the contribution of adrenalin stimulated lipolysis and the concomitant intracellular reesterification of released fatty acids to the final NEFA output from the adipose tissue in non obese hereditary hypertriglyceridemic rats [HHTg], genetically fixed model of insulin resistance and control [C] normotriglyceridemic rats in vitro. The separate set of experiments was designed to elucidate the mechanism of adrenalin stimulated transport of glucose into the adipocytes.
Materials and Methods: C and HHTg (weight 300 ± 20 g) rats were fed two weeks high sucrose (HS,70% of calories as sucrose) diet. The in vitro experiments were carried out after overnight fasting. The distal parts of epididymal adipose tissue were incubated either in basal Krebs-Ringer buffer (with 5 mmol/l glucose, 2% albumin and 0,1 uCi 14C-glucose/ml) or in the basal medium supplemented with adrenalin (1 mg/ml), wortmannin (1 mM) or adrenalin + wortmannin and the glycerol and NEFA release. was determined. The utilization of glucose for esterification was assessed according to the incorporation of 14C-glucose into glycerol-glyceride of lipids.
Results: HS feeding increased postprandial serum Tg by 90% in HHTg and by 21% in C. Fasting FFA raised after the administration of the diet from 1 ± 0,08 to 1,3 ± 0,07 mmol/l in HHTg but did not change in C (0,79 ± 0,03 and 0,78 ± 0,04 mmol/l). The adrenalin stimulated lipolysis measured as glycerol release was lower in HHTg compared with C (7,2 ± 0,5 vs 11,8 ± 1 mmol glycerol/g w.wt., p < 0,05) but the NEFA release was comparable in both groups (9 ± 0,73 vs 9,3 ± 0,3 umol NEFA/g w.wt.). Adrenalin stimulated the incorporation of glucose into glycerol-glyceride in C (1,6 ± 0,1 vs 3 ± 0,2 mmol/g w.wt., p < 0,01) but had no effect in HHTg. In the separate experiments in controls the PI-3 kinase was inhibited by wortmannin. Wortmanin has no effect on neither basal (3,7 ± 0,2 vs 3,7 ± 0,3 mmol glycerol/g w.wt., n.s.) nor adrenalin stimulated (11,8 ± 1,5 vs 14 ± 1 mmol glycerol/g w.wt., n.s.) lipolysis measured as glycerol release but increased the NEFA release into the medium (9,1 ± 1,5 vs 18,6 ± 1,2 mmol NEFA/ g w.wt., p < 0,001). In contrast, wortmannin significantly decreased the adrenalin stimulated incorporation of glucose into glyceride-glycerol (3,0 ± 0,2 vs 1,7 ± 0,05 mmol glucose/g w.wt., p < 0,001).
Conclusion: Our results indicate that even in the situation of stimulated lipolysis up to 50% of NEFA released by hydrolysis of triglycerides is reesterified and render in the form of lipids inside the adipocyte. The application of wortmannin showed that the prevention of NEFA reesterification increased the NEFA release from adipose tissue by 100%. We conclude that the actual amount of NEFA released from adipose tissue is determined not only by the lipolysis but also by their reesterification and entrapment in the adipose tissue. In the insulin resistant HHTg rats the lower availability of glucose for reesterification may lead to the increased NEFA concentration in the circulation. The results obtained using the specific inhibitor of PI-3 kinase wortmannin indicated the involvement of PI-3 kinase in the adrenalin stimulated transport of glucose into the cells of white adipose tissue. It opens the question of possible convergence of insulin- and adrenalin stimulated pathway of glucose transport in adipose tissue.
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Adipose tissue fatty acid metabolism and the metabolic syndrome
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Background and Aims: The metabolic syndrome is a potent cause of excess cardiovascular morbidity and mortality. The pathophysiology underlying the dyslipidaemia of the metabolic syndrome is poorly understood. Of particular interest is the possibility that the metabolic syndrome is associated with abnormal fatty acid (FA) handling within adipose tissue. In addition, little is known as to whether FA handling is dependent on the source of FA and whether there are alterations in the handling of dietary derived or

endogenous FAs in the metabolic syndrome. We hypothesised that reduced trapping of dietary derived FAs in adipose tissue results in increased exposure of peripheral tissue to FAs
Materials and Methods: Insulin resistant subjects (n=8) were compared with age and body mass index (BMI)-matched controls (n=8). Subjects received an infusion of [$^2H_2$]palmitic acid and ingested [U-^{13}C]palmitic acid in a mixed meal. The [$^2H_2$]palmitic acid mixes with the non-esterified fatty acid (NEFA) pool and is used to trace the fate of endogenous FAs. The [U-^{13}C]palmitic acid is incorporated into chylomicrons and allows assessment of the fate of dietary derived FAs. Blood samples were taken prior to meal ingestion and at intervals over 6 hours. Isotopic enrichment of NEFA and triacylglycerol (TG) was measured by gas chromatography-mass spectrometry
Results: Plasma insulin concentrations in the subjects with the metabolic syndrome were double those of controls during both the fasting and postprandial periods. Unexpectedly, NEFAs were not elevated in the metabolic syndrome subjects, indeed there was a trend for lower fasting NEFAs in this group. Furthermore, NEFA output from adipose tissue was significantly lower during fasting in the metabolic syndrome (p=0.015). In the whole study group there was a negative correlation between fasting insulin and fasting adipose tissue NEFA output (r=-0.81, p=0.001). In addition, during the postprandial period, NEFA output from adipose tissue suppressed very little further in the metabolic syndrome subjects. A proportion of the ^{13}C labelled NEFA, derived from chylomicron hydrolysis, spilled out of adipose tissue into the systemic circulation. There was no difference in this spillover fraction between the two groups, implying no difference in the trapping of dietary derived fatty acids. $^2H_2$ labelled NEFA, a tracer of circulating NEFA, demonstrated net uptake into adipose tissue equally in the two subject groups during both the fasting and postprandial periods. This implies mixing of FAs from the circulating NEFA pool with those derived from plasma TG lipolysis prior to adipose tissue uptake.
Conclusion: We found no evidence of reduced trapping of dietary derived FAs or increased circulating FAs in subjects with the metabolic syndrome. The suppressed NEFA output during fasting and the relationship with insulin implies that, in the metabolic syndrome, adipose tissue retains sensitivity to prevailing insulin concentrations and is not insulin resistant. The blunted postprandial response suggests a metabolic inflexibility in the face of changing metabolic conditions. Although there was no difference in the handling of the FA tracers between the two groups, the finding of a net uptake of endogenous FAs within adipose tissue, regardless of metabolic conditions, is novel and intriguing.
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Distinct pattern for the desaturase- and elongase-catalyzed steady-state equilibrium between selected fatty acids in the liver phospholipids and triglycerides of animals with either type 1 or type 2 diabetes
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Background and Aims: Diabetes is known to affect the concentration and/or composition of lipid components in different organs. The major aim of the present study was to compare the liver content of phospholipids and triglycerides and their fatty acid pattern in two animal models of diabetes, namely in rats injected at the adult age with streptozotocin (type 1 diabetes) and in Goto-Kakizaki rats (type 2 diabetes)
Materials and Methods: Male STZ-injected rats (257 ± 9 g body wt; 19.2 ± 2.4 mM glycemia) and GK rats (324 ± 4 g body wt; 11.1 ± 0.8 mM glycemia) given free access to the same food and water were examined for their liver content in phospholipids and triglycerides as well as the composition of these lipids in different fatty acids
Results: The liver phospholipids content was comparable in STZ and GK rats, whereas the liver triglyceride content was 2–3 times higher in GK than STZ rats. The C16:0/C16:1ω7 ratio was twice higher in both the phospholipids and triglycerides of STZ rats, as compared to GK rats. The C18:0/C18:1ω9 ratio was also higher in the triglycerides of STZ rats, whilst an opposite situation prevailed in liver phospholipids. The C18:2ω6/C18:3ω6 ratio was significantly higher in the phospholipids and the triglycerides of GK than STZ rats, whilst the opposite situation prevailed in the case of the C18:3ω6/C20:4ω6 ratio. Concerning elongase-catalysed reactions, the C20:4ω6/C22:4ω6 ratio was significantly higher in both the phospholipids and triglycerides of STZ, as compared to GK, rats. Surprisingly, however, no significant difference was observed when comparing the (C16:0 + C16:1ω7)/(C18:0 + C18:1ω9) paired ratio in either liver phospholipids or triglycerides of STZ versus GK rats. Comparable findings were made for the same fatty acids in plasma triglycerides and phospholipids. For instance, in the GK rats, the correlation coefficient between liver and plasma fatty acid pattern (logarithmic scale) amounted to 0.986 (p<0.001)
Conclusion: The present findings document striking differences between STZ and GK rats in terms of both the liver triglyceride content and pattern for desaturase- and elongase-catalysed steady-state equilibrium between selected fatty acids in liver phospholipids and triglycerides. With the exception of the C18:0/C18:1ω9 ratio in liver phospholipids, the results suggest increased activity of Δ9-desaturase in the insulin resistant GK rats. However, an opposite situation prevailed for the Δ6-desaturase and, apparently, also for the Δ5-desaturase. Although the latter finding would again be compatible with insulin resistance in the GK rats, its significance may be obscured by a higher activity of elongase in these GK rats, as suggested by the C20:4ω6/C22:4ω6 but not the (C16:0 + C16:1ω7)/(C18:0 + C18:1ω9) ratio. The present findings thus point to a multifactorial regulation of the concerned ratios with a tight correlation between liver and plasma values

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Insulin treatment increases hepatic intracellular lipids by activating fatty acid synthase and inhibiting fatty acid oxidation in type 2 diabetes rats
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Background and Aims: Type 2 diabetes are frequently associated with increased lipid content in no-fat tissue such as liver and muscle due to the insulin resistance, a condition which composes of multidisorder in lipid and glucose homeostasis such as hyperinsulinemia and hyperlipidemia. However, since the impaired insulin signaling, the increased insulin secretion is still unable to normalize the pathological enhanced blood glucose in most type 2 diabetes. So, clinically, insulin therapy is a commonly accepted strategy nowadays for those who have a much high glucose level in order to attenuate the glucotoxicity. As it is wildly known that insulin is a lipogenesis hormone, it is emerging with respect to if insulin treatment on type 2 diabetes will increase fat accumulation in liver and worsen the possible existed fatty liver in these patients. This study was therefore designed to examine the effect of insulin treatment on hepatic intracellular lipid content and lipid metabolism in liver in high-fat induced type 2 diabetes rats and the possible mechanism behind the fat accumulation.
Materials and Methods: Male wistar rats weighing 140–160 g were treated by the streptozotocin (30 mg/kg) and high-fat diet to establish type 2 diabetes models and these rats were randomly divided into two groups: untreated group (UT) and insulin treated group (IT). The normal rats of same breed were included as control (NC). The treatment of each group with either NPH insulin (4 U/kg/day), or saline continued for 4 weeks. Body weight, blood glucose, insulin, plasma and hepatic intracellular lipid profile, hepatic fatty acid oxidation and the activity of fatty acid synthase (FAS) were detected.
Results: Comparing with NC group, the hepatic intracellular and plasma TG,TC and FFAs were increased significantly in UT group (P<0.05), and lipid droplets could be seen dispersingly in the liver specimens, the hepatic fatty acid oxidation were increased markedly (P<0.05) while the fatty acid synthase activity decreased (P<0.05). Insulin treatment resulted in a further accumulation of lipids in liver by 55.7%, 19.87% and 22.2% increase in TG, TC, FFAs respectively and the size of hepatocyte enlarged and filled with fat. While plasma TG and TC did not show much difference after the insulin treatment, although still significant higher than NC. Hence, contrast to what was seen in liver, plasma FFAs in IT decreased noticeblely by 11% (IT vs UT, P<0.05). Meanwhile, insulin treatment was companied by 20% decrease in the rate of fatty acid oxidation and 31% increase in hepatic FAS activity comparing to UT.
Conclusion: The results of this study indicates that administration of insulin to type 2 diabetes could aggravate of fatty liver, which may exist before the insulin application. Moreover, insulin induced lipids deposit in liver is at least partly by acting on inhibiting fatty acid oxidation and activating FAS.

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Incorporation of exogenous and endogenous fatty acids into very low-density lipoprotein (VLDL): a study using stable isotope and immunoaffinity techniques in humans

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Background and Aims: Dietary fatty acids enter metabolic pathways in the body where they mix with endogenous fatty acids. Chylomicron remnants and endogenous fatty acids may be taken up by the liver and used for the synthesis of very low-density lipoprotein (VLDL). Current knowledge regarding the mixing of these pools in vivo is limited. The objective of the present study was to investigate the incorporation of exogenous and endogenous fatty acids into VLDL by using fatty acid stable isotope and apolipoprotein immunoaffinity techniques in humans.
Materials and Methods: Eight healthy males were given an intravenous infusion of fatty acid tracer ([$^2H_2$]palmitic acid complexed with albumin) to investigate endogenous non-esterifed fatty acid (NEFA) incorporation into VLDL. Participants were also fed a mixed meal containing [U-^{13}C]palmitic acid to investigate the incorporation of exogenous fatty acids into VLDL. Blood samples were taken at 0, 2, 3, 4, 5 and 6 hours postprandially. Triacylglycerol rich lipoprotein particles were isolated by density gradient ultracentrifugation (Svedberg flotation rate S_f 20-400). Separation of VLDL was performed using immunoaffinity separation of particles containing Apolipoprotein B100 (Apo B100). Lipids were extracted from plasma using chloroform-methanol (2:1 v/v) and plasma NEFA were separated using solid phase extraction. Isotopic enrichment of [$^2H_2$]palmitic acid and [U-^{13}C]palmitic acid in VLDL and plasma NEFA was measured by gas chromatography-mass spectrometry.
Results: Enrichment of [$^2H_2$]palmitate into VLDL was rapid and the contribution of plasma NEFA to VLDL-triacylglycerol appeared to be in the range of 45%± 15% (mean±standard deviation). There was a significant delay ($p<0.002$) in the peak enrichment of [$^2H_2$]palmitate in VLDL compared with plasma NEFA, indicating an expected delay in the export of fatty acids as VLDL-triacylglycerol. A steady state of [$^2H_2$]palmitate enrichment into VLDL was reached within 6 hours. The appearance of exogenous fatty acids from the meal ([U-^{13}C]palmitic acid) into VLDL was also rapid. The shape of the curves for [$^2H_2$]palmitate and [U-^{13}C]palmitic acid incorporation into VLDL and the accumulation of the isotopic tracers suggests that there is mixing of the endogenous and exogenous fatty pools that contribute to VLDL-triacylglycerol.
Conclusion: The present study demonstrates the use of fatty acid stable isotopes and apolipoprotein immunoaffinity techniques to investigate endogenous and exogenous fatty acid incorporation into VLDL in humans. These techniques can now be used to investigate whether there are disturbances in insulin resistance that may explain partitioning of different pools of fatty acids in the liver for VLDL secretion.
Support: The Wellcome Trust, The Food Standards Agency, Novo Nordisk and Girdlers'/HRC New Zealand

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AMPK activation by AICAR decreases respiratory quotient and lowers plasma and tissue triglyceride levels in obese fa/fa rats

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Background and Aims: 5'-AMP-activated protein kinase (AMPK) plays a critical role in the regulation of cellular energy homeostasis. The AMPK activator 5-aminoimidazole-4-carboxamide-riboside(AICAR) has been shown to reduce hyperglycaemia, improve insulin sensitivity and alleviate hyperlipidaemia in rodent models of diabetes and obesity. It is however unknown if AICAR impacts indices of energy metabolism, namely respiratory quotient (RQ) and energy expenditure (EE). Here, we assessed whether AICAR affects RQ or EE in obese fa/fa rats. In addition, we determined plasma and tissue triglyceride (TG) levels.
Materials and Methods: Male fa/fa rats (n=5) were treated for 5 days with vehicle or AICAR (250 and 500 mg/kg/qd./sc.). During the experiment RQ and EE were measured continuously using indirect calorimetry. At day 5, ACC phosphorylation in liver and EDL-muscle was measured by western blotting and organ TGs were determined. To measure LDL synthesis, HepG2 hepatoma cells were incubated with AICAR and 0.4 µCi [^{14}C]acetate. Radioactive LDL was extracted from the medium. To measure lipid oxidation, clone 9 rat hepatocytes were incubated with AICAR and 0.4 µCi [^{14}C]oleic acid. Liberated [^{14}C] CO_2 was trapped with 40% KOH.
Results: AICAR dose-dependently reduced the RQ (control: 0.98; 250 mg/kg AICAR: 0.94; 500 mg/kg AICAR: 0.89), indicating a shift from carbohydrate oxidation towards fat oxidation. During the course of the experiment EE was temporary decreased by the treatment (250 mg/kg AICAR: -4J/gh; 500 mg/kg AICAR: -10J/gh), while body weight was not influenced. At the end of treatment, AICAR reduced TG content in liver (control: 1.4 ± 0.8; 250 mg/kg AICAR: 1.2 ± 0.5; 500 mg/kg AICAR: 0.5 ± 0.1 mM/g tissue, $p<0.05$) and muscle (control: 31.4 ± 9.5; 250 mg/kg AICAR: 15.9 ± 2.9, $p<0.01$; 500 mg/kg AICAR: 20.0 ± 8.5 mM/g tissue) as well as TGs in blood (control: 2.9 ± 0.8 mM; 250 mg/kg AICAR: 2.0 ± 0.2 mM, $p<0.05$; 500 mg/kg AICAR: 1.1 ± 0.2 mM, $p<0.001$). In vitro, AICAR (1 mM) reduced LDL-synthesis by 48.2% ($p<0.002$) and increased ^{14}C-oleate oxidation by 171.9% ($p<0.0001$) in hepatocyte cell lines.
Conclusion: The results indicate that AMPK activation by AICAR reduces ectopic lipid accumulation in fa/fa rats by increasing lipid oxidation, leading to a decrease in RQ. This is accompanied by a temporary decrease in EE, of which the underlying mechanism has to be clarified. Pharmacologic AMPK activation may contribute to improving the treatment of the metabolic syndrome by affecting lipid as well as glucose metabolism.

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Association of β_2 adrenergic receptor polymorphisms and related haplotypes with triglicerides and LDL-cholesterol levels

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Background and Aims: Adrenergic receptors regulate lipid mobilization, energy expenditure and glycogen breakdown. The β_2 adrenergic receptor (β_2-AR) gene may constitute a potential candidate gene to explain part of the genetic predisposition to human obesity and correlated traits. With regard to the association between β_2-AR gene polymorphisms and obesity related metabolic disorders, published reports give conflicting results. We investigated the role of three polymorphisms, and related haplotypes of the β_2-AR in the obesity and related traits in a cohort of overweight/obese subjects.
Materials and Methods: We characterized one single nucleotide polymorphism (SNP) in the promoter region (5'LC-Cys19Arg) and two in the coding region (Gly16Arg and Gln27Glu) of the β_2-AR in 642 consecutively recruited overweight/obese subjects on whom extensive clinical and biochemical analyses were performed. The three missence mutation of β_2-AR gene, were genotyped using the flurogenic 5' nuclease assay application of the ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems). The effect of the polymorphisms on quantitative variables was investigated using multiple linear regression analysis. We created two dummy variables to regress the three genotypes of each SNP. To perform Haplotype analysis we used THESIAS program.
Results: 5'LC-Cys19 homozygous showed higher levels of triglycerides and LDL-cholesterol compared to 5'LC-Arg19 homozygous ($p=0.03$ and $p=0.01$, respectively). Similar increase in triglycerides and LDL-cholesterol levels was observed for Arg/Arg genotype compared to Gly/Gly genotype of Gly16Arg polymorphism ($p=0.02$ and $p=0.01$, respectively) and for Gln/Gln genotype compared to Glu/Glu genotype of the Gln27Glu polymorphism ($p=0.01$ and $p=0.03$, respectively). The 5'LC-Cys^{19}Arg^{16}Gln^{27} haplotype determined a significant increase in triglyceride and LDL-cholesterol levels compared to 5'LC-Arg^{19}Gly^{16}Glu^{27} haplotype ($p=0.05$ and $p=0.02$, respectively).
Conclusion: Consistently with the evaluation of single SNPs, our observations prove that 5'LC-Cys^{19}Arg^{16}Gln^{27} haplotype of the β_2-AR gene, confers higher triglycerides and LDL-cholesterol levels in overweight/obese subjects compared to 5'LC-Arg^{19}Gly^{16}Glu^{27} haplotype carriers.
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PS 49

Action of other hormones

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Molecular basis of ligand binding selectivity by the insulin and IGF-I receptors

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Background and Aims: The structure-function relationships of insulin and IGF-I binding to their physiological receptors (members of the receptor tyrosine kinase superfamily) have been studied for more than three decades using a variety of approaches, including photoaffinity crosslinking, chimeric receptors, and site-directed mutagenesis of ligands and receptors, but we still have a very incomplete understanding of the molecular basis of high affinity ligand binding selectivity of the two receptors (insulin receptor A- and B-isoforms and IGF-I receptor). We have embarked on a systematic program of single and double amino acid mutagenesis (including alanine scanning) of the insulin molecule, as well as reciprocal substitutions between insulin, IGF-I and IGF-II, and study of selected mutations in IGF-I.
Materials and Methods: The mutations were introduced to the expression vector by site-directed mutagenesis. The analogues were expressed in *S. cerevisiae* using the expression vector pJB146 from Novo Nordisk. The expressed analogues were tested for binding affinity and negative cooperativity in the human IM-9 lymphocyte cell line, which expresses the A-isoform of the insulin receptor. Selected IGF-I analogues were purchased from Gropep and their affinity and negative cooperativity tested in NIH3T3 fibroblasts overexpressing the IGF-I receptor.
Results: We previously reported that systematic alanine scanning mutagenesis of the insulin molecule confirmed the importance of residues involved in insulin dimerization, as postulated more than three decades ago, but also mapped a novel binding surface overlapping with the surface involved in hexamerization. Double mutations in either each of these surfaces or in both surfaces showed an additional impairment in binding affinity, sometimes resulting in complete loss of binding. No mutation outside the previously reported "cooperative site" resulted in loss of negative cooperativity in insulin binding, with the exception of an insulin analogue with residues A1-4 removed which behaved as an antagonist for negative cooperativity, while analogues with individual substitutions at either A1,2,3 or 4 were agonistic for this property. Removing A1-4 may perturb a cooperative interaction between A2 and A3 and residues at the end of the B chain previously shown to be critical for negative cooperativity. No mutation in IGF-I has yet revealed a loss of negative cooperativity. Substitution of the residues that differ in IGF-I and IGF-II in the insulin molecule showed that 4 substitutions in the A- and B-domains of the IGFs (at A8, A10, B5 and B16) largely explain the low affinity of the IGFs for the insulin receptor A-isoform. Two substitutions in IGF-II that increase binding to the insulin receptor when introduced in insulin explain the higher affinity of IGF-II for the insulin receptor A-isoform.
Conclusion: Our data support a model in which two surfaces of the insulin molecule create high affinity by crosslinking two distinct domains on the insulin receptor alpha subunits. The substitutions in IGF-I and IGF-II that explain the lower affinity for the insulin receptor are localised at two distinct spots in the equivalent of the insulin "classical" (dimerization) binding surface. The low affinity of insulin for the IGF-I receptor likely results from the lack of an equivalent to the IGF-I C-peptide.

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HPA axis regulation in type 1 diabetes

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Background and Aims: Diabetes mellitus is associated with various cerebral disturbances in both humans and animals. Using the pharmacological model of type 1 diabetes, streptozotocin (STZ)-treated mice, we previously described that hippocampal astrocytes and neurons are strongly activated one month after diabetes induction, exhibiting also higher oxidative stress markers. Based on these results and the involvement of the hippocampus in stress-system regulation via the Hypothalamus-Pituitary-Adrenal (HPA) axis, we now examine central and peripheral markers of HPA axis activity in diabetic mice.
Materials and Methods: Three months old male C57BL/6J mice were injected with STZ (170 mg/kg i.p.) or vehicle. Every other day blood samples were taken via tail incision to determine corticosterone, ACTH and glycemia levels. At the same time, body weight and food and water intake were measured. After 14 days, mice were decapitated, brain sections were processed for *in situ* hybrization.
Results: While plasma corticosterone was elevated from day 1 to day 14 after the onset of the disease, ACTH was significantly decreased after 14 days of diabetes (diabetic=70.94 ± 8.03 ng/ml; n=6, control=158.4 ± 20.31 ng/ml; n=7, $p<0.01$). MR mRNA expression in the dentate gyrus of the hippocampus was significantly decreased in diabetic mice (diabetic= 58.88 ± 3.32, control=69.69 ± 3.57; n=8, $p<0.05$). GR mRNA expression in the hippocampus and the paraventricular nucleus of the hypothalamus as well as CRH mRNA in the latter region were similar in both groups.
Conclusion: The augmented corticosterone secretion occurs one day after the onset of diabetes in a condition of increased glycemia, indicating that the rise in blood glucose levels (or its consequences) are responsible for the increased corticosterone. Decreased MR mRNA in relation to decreased ACTH release in the face of exaggerated corticosterone secretion suggest a profound disturbance in HPA axis regulation, most likely including sensitization of the adrenals to ACTH. If these changes in HPA axis regulation contribute to cognitive dysfunction and an impaired ability to respond to stress will be the topic of future studies.
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Effects of nutrient ingestion on splanchnic cortisol production in humans

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Background and Aims: The splanchnic bed produces cortisol at rates approximating extra-adrenal tissues by converting cortisone to cortisol via the 11 β-hydroxysteroid dehydrogenase type1 (11 β-HSD-1) pathway. It presently is not known whether splanchnic cortisol production is regulated by nutrient ingestion and/or by the accompanying changes in hormone secretion.
Materials and Methods: To address this question, 18 healthy humans matched for age, sex, BMI, %body fat, and lean body mass were randomized to ingest either a mixed meal consisting of ~500 calories,45% carbohydrate, 15% protein, and 40% fat (n=10) or to a saline infusion (n=8) while total body, splanchnic and D-3 cortisol production (an index of 11 β-HSD-1 activity) were measured using the combined hepatic catheterization and D-4 cortisol infusion methods.
Results: Fasting glucose and insulin concentrations did not differ on the meal and saline study days. Glucose and insulin concentrations increased following meal ingestion peaking at 11.0 ± 1.0 mmol/l and 451 ± 64 pmol/l respectively at 45 minutes then fell to baseline thereafter. In contrast, glucose and insulin concentrations slowly fell to 5.1 ± 0.1 mmol/l and 27 ± 6 pmol/l during the 6 hours of observation on the saline study day. Fasting cortisol concentration did not differ on the meal and saline study days. Cortisol increased ($p<0.05$) to a peak of 353 ± 55 nmol/l after meal ingestion but did not change after saline ingestion. The increase in cortisol following meal ingestion was associated with an increase in both total body cortisol (from 748 ± 63 to1620 ± 235 nmol/min; $p<0.01$) and total body D-3 cortisol (from 99 ± 11 to143 ± 11 nmol/min; $p<0.01$) production whereas there was no change in either on the saline study day. The increase in total body cortisol and D3-cortisol production following meal ingestion originated in extra-splanchnic tissues since splanchnic cortisol production (mean 0–360 min: 254 ± 83 vs.262 ± 36 nmol/min), and splanchnic D-3 cortisol production (mean 0–360 min: 72 ± 22 vs.77 ± 14 nmol/min) did not differ on the meal and saline study days
Conclusion: Mixed meal ingestion does not alter either splanchnic cortisol production or the conversion of D4-cortisol to D3-cortisol, and therefore by implication, flux via the splanchnic 11 β-hydroxysteroid dehydrogenase type 1 pathway. Thus, the increase in plasma cortisol concentrations observed after ingestion of a mixed meal originates in extra-splanchnic tissues.
Support: NIDDK

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Negligible splanchnic conversion of cortisol to cortisone via the 11β-HSD type 2 pathway occurs in normal humans

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Background and Aims: By using hepatic catheterisation along with D_4-cortisol infusion, it has been recently shown that a considerable amount of cortisol is produced in the splanchnic bed from cortisone via 11β-hydroxysteroid dehydrogenase (11β-HSD) type 1 conversion. Since only cortisol data were analysed, no information was derived on the presence, in the same region, of 11β-HSD type 2, able to convert cortisol to cortisone. The purpose here is to assess cortisol and cortisone kinetics within the splanchnic bed, in order to quantify the rates of splanchnic conversion of cortisone to cortisol and of cortisol to cortisone, and the rates of utilizations of the two hormones.
Material and Methods: 10 lean non diabetic subjects underwent a primed continuous infusion of D_4-cortisol (0.22 mg prime, 0.19 mg/h continuous). After 210 min equilibration period, four samples were taken, 10 min apart, from catheters in femoral artery and hepatic vein for measurement of unlabelled and labelled cortisol and cortisone. An infusion of indocyanine green dye was also given and the arterial hepatic venous gradient of the dye was measured at the four sampling times for determination of hepatic blood flow. The study was performed twice, first at basal glucose and insulin concentrations and then during an euglycemic hyperinsulinemic clamp. A new model of cortisol-cortisone interactions at regional level was developed. Because D_4-cortisol loses a deuterium during conversion to D_3-cortisone, model identification is based on cortisol, cortisone, D_4-cortisol, D_3-cortisol and D_3-cortisone data and provides estimates, in each subject, of interconversion fluxes: cortisone to cortisol (F_1) and cortisol to cortisone (F_2) and utilization "per se", i.e. without resorting to interconversion, of cortisol (U_1) and cortisone (U_2). All fluxes are given as mean±SE and expressed as μg/min.
Results: Net splanchnic balance indicates a net release of cortisol in the basal state (-7.97 ± 3.37) which becomes negligible during insulin infusion (-0.89 ± 4.78) and a net utilization of cortisone, both before (21.58 ± 4.87) and during (19.95 ± 4.93) insulin infusion. When the model is exploited to segregate net balances in interconversion and utilization rates of the two hormones, average values of model fluxes are, in the basal state: F_1=21.23 ± 3.16; F_2= -0.21 ± 0.20; : U_1=13.19 ± 3.49; U_2= 1.62 ± 0.81 and during insulin infusion: F_1=19.38 ± 3.74; F_2= -0.04 ± 0.25; : U_1=19.49 ± 5.94; U_2= 2.64 ± 4.23.
Conclusion: Our results indicate a consistent production of cortisol but not of cortisone in the splanchnic bed, thus suggesting that 11β-HSD type 1 is expressed in this region, but not 11β-HSD type 2. Cortisol is utilized "per se" in the splanchnic bed, and insulin infusion induces a modest increase in this flux, while cortisone is not utilized "per se", but only after conversion to cortisol.

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Dynamic testing of growth hormone/insulin-like growth factor-1 axis in type 2 diabetes mellitus

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Background and Aims: Growth hormone (GH)/insulin-like growth factor-1 (IGF-1) axis contributes mitogenic, atherogenic and proliferative process of the body. Hypopituitarism associated with diabetes mellitus (DM) was defined to have a protective role against proliferative complications of DM. Evenly, pituitary radiotherapy was offered by some authors in the past, as a therapeutic option for proliferative retinopathy. We aimed to investigate GH/IGF-1 axis in type 2 DM in a cross-sectional case-control study.
Materials and Methods: Fourty-six type 2 diabetic cases (M/F: %58, age: 55.5 ± 10.6 yrs, BMI: 30.8 ± 7.2 kg/m2, disease duration: 4.82 ± 4.73 yrs, HbA1c: % 7.4 ± 1.5, FPG: 167.7 ± 59.6 mg/dl, PPG: 208.9 ± 97.4 mg/dl) were matched with 28 healthy controls according to age, sex, and body-mass-index. Patients with proliferative complications were excluded. Overnight fasting samples were obtained for basal GH and IGF-1 levels. The day after, IGF-1 generation test was performed with single dose injection of GH 0.1 mg, s.c., at 08 00 am. Response to exogenous GH was evaluated via IGF-1 assays at 24th hour of injection [ΔIGF-1= (Stimulated IGF-1) - (Basal IGF-1)].
Results: Basal GH (0.28 ± 0.24 ng/ml vs 0.49 ± 0.52 ng/ml) and IGF-1 (207.8 ± 102.0 mcg/l vs 278.9 ± 131.6 mcg/l) levels were significantly lower in patients with type 2 DM, compared to controls, (p values: 0.017 vs 0.011, respectively). Stimulated IGF-1 levels (377.3 ± 143.9 mcg/l vs 487.6 ± 135.4 mcg/l, respectively in cases and controls) were also lower in type 2 DM (ΔIGF-1: 166.8 ± 63.6 mcg/l) than controls (ΔIGF-1: 208.7 ± 41.4 mcg/l), (p=0.002). Patients with poor glycemic control (HbA1C > % 8.5) tended to give a lower response to IGF-1 generation test (ΔIGF-1: 144.5 ± 67.5 mcg/l) than the patients (HbA1C < %7.0) with good glycemic control (ΔIGF-1: 180.3 ± 66.4 mcg/l), but the difference did not reach statistical level of significance (p=0.187). In contrast, presence of current insulin therapy (122.5 ± 53.3 mcg/l vs 180.6 ± 60.8 mcg/l) and micro- (115.6 ± 51.7 mcg/l vs 180.7 ± 59.8 mcg/l) or macrovascular (135.5 ± 65.6 mcg/l vs 180.8 ± 58.5 mcg/l) complications were associated with decreased ΔIGF-1 levels at a statistically significant level (p values: 0.01, 0.006, and 0.008 respectively), compared to lack of each conditions respectively.
Conclusion: Our study suggests that, type 2 DM is associated with a decrease in basal and stimulated IGF-1 levels. Ongoing exogenous insulin therapy and presence of micro- or macrovascular complications display a further decrease in the IGF-1 response to exogenous GH administration, but glycemic control do not. Implications of our findings on the glycemic-control independent complications of diabetes require further clinical trials.

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Up-regulation of mitochondrial biogenesis and β-oxidation in white fat by ω-3 polyunsaturated fatty acids of marine origin

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Background and Aim: Polyunsaturated fatty acids (PUFA) of marine origin limit accretion of body fat and improve symptoms of metabolic syndrome. Aim of this work was to investigate in mice whether the stronger effect of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid on adiposity, when compared to their precursor α-linolenic acid (ALA), could involve modulation of gene expression and metabolism in the white fat.
Materials and Methods: Male 4 mo-old C57BL/6J mice fed high-fat (HF; containing 20% lipids) diet with or without admixed EPA/DHA, and cultures of 3T3-L1 adipocytes incubated in the presence of various PUFA were used in the study. Oligonucleotide microarrays, cDNA PCR-subtraction, and quantitative real time RT-PCR were used to identify genes modulated by EPA/DHA. Immunoblotting analysis and measurements of cytochrome oxidase (COX) activity were used to validate the results on the protein level. Effect of EPA/DHA on fatty acid (FA) oxidation and synthesis was also examined.
Results: Replacement of 44% of dietary lipids in the HF diet by EPA/DHA concentrate resulted in 11 ± 2% decrease in body weight (means±SE of 4 independent experiments, n = 7–11) after 4 wk treatment. Weight of epididymal fat was 30 ± 3% lower (compared to HF-fed group), weight of dorsolumbar fat did not change significantly. Food intake was not affected.
Using cDNA PCR-subtraction and oligonucleotide microarray analysis, we identified a subset of genes, the expression of which was affected by the EPA/DHA treatment. In general, we observed up-regulation of genes coding for components of mitochondrial respiratory chain and down-regulation of genes promoting lipogenesis. Real time RT-PCR revealed approximately 3-fold induction of key regulators of mitochondrial biogenesis, PPAR-γ coactivator-1 (PGC-1) and nuclear respiratory factor-1 (NRF-1), predominantly in epididymal white fat. Expression of carnitine-palmitoyl transferase 1 (which regulates FA entry into mitochondria) and acyl-CoA oxidase 1 (marker of peroxisomal FA oxidation) was also increased. All these results were supported by analogous measurements in white fat depots from animals fed another type of HF diet, containing as much as 40% lipids with or without partial replacement by EPA/DHA, in isolated adipocytes (in order to eliminate the impact of stromal vascular cells), and in 3T3-L1 cells treated with mixture of oleate and ALA or DHA, respectively.
In agreement with the mRNA findings, we observed approximately 2-fold increase in 70 kDa subunit of succinate dehydrogenase, COX1, COX6, and α subunit of F_1-ATPase proteins in crude cell membranes, and 1.5 to 1.8-fold higher oxidation of oleate (when normalized to tissue weight, protein, or DNA content) and 1.4-fold lower incorporation of ^3H_2O into saponifiable FA (related to DNA content) in fragments of epididymal fat of EPA/DHA treated animals.
Conclusion: Increase in mitochondrial biogenesis and FA oxidation, and decrease in fatty acid synthesis in adipocytes contribute to the anti-adipogenic effect of EPA and DHA.
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Dietary-induced alterations in mitochondrial oxidative phosphorylation (OXPHOS) functions in muscle rats. Association with induction of glucose intolerance

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Background and Aims: Insulin resistance in skeletal muscle is a major factor in the pathogenesis of type 2 diabetes. It precedes the clinical development of the disease by 10 to 20 years and has been related to decreased muscle mitochondrial oxidative capacity. This study aimed at characterizing the abnormalities of the mitochondrial energy metabolism, i.e. OXPHOS, associated with the induction of insulin resistance using 5 dietary strategies in 3-month old male Wistar rats.
Materials and Methods: Animals were fed during 6 weeks with 3 normocaloric diets: control (NCD), 19%-sucrose (NSD) and 45%-fat (NFD), or with 2 hypercaloric diets: 19%-sucrose (HSD) and 45%-fat (HFD). Glucose intolerance was evaluated using intraperitoneal G50 injection. Mitochondrial oxygen consumption (state 2 without ADP, state 3 with ADP 1 mM, RCR = state 3/state 2) and ATP production (vATP, ATP/O = vATP/state 3) were measured on permeabilized fibers from oxidative (soleus) and glycolytic (tibialis) muscles using glutamate/malate and succinate as substrates of complex I and II, respectively.
Results: Glucose intolerance was increased by 61% in NFD ($P<0.05$), and by 106–148% in NSD, HFD and HSD ($P<0.001$), compared to NCD. In soleus muscle, complex I state 2, state 3, and vATP were significantly and gradually decreased with glucose intolerance ($P<0.001$), while RCR and ATP/O were unchanged in the 5 groups. Complex II state 2 and state 3 were significantly decreased in NSD, HFD and HSD ($P<0.001$), but RCR, vATP and ATP/O were unchanged in the 5 groups. In tibialis, complex I state 2 and state 3 were unchanged in the 5 groups while vATP and ATP/O were significantly decreased in NSD, HFD and HSD ($P<0.05$). Complex II state 2, state 3, RCR, vATP and ATP/O were unchanged in the 5 groups.
Conclusion: Dietary-induced glucose intolerance is associated with muscle-specific alteration of complex I activity, the result being a reduction vATP in both muscle. By contrast, complex II vATP is not altered in either muscle, despite decreased state 2 and state 3 in soleus.

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High-fat diet-induced remodeling of adipose tissue in obese diabetic mice is abrogated by polyunsaturated n-3 fatty acids

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Background and Aims: White adipose tissue is progressively infiltrated by macrophages as obesity develops. Matrix metalloproteinases (MMPs) and cathepsin K are enzymes essential for extracellular matrix degradation and growth of adipose tissue. MMP-12 is required for macrophage-mediated proteolysis and tissue invasion. Since polyunsaturated fatty acids (PUFA) particularly of the n-3 series show anti-inflammatory actions we aimed at investigating how PUFA influence matrix remodeling of white adipose tissue in obesity.
Materials and Methods: Male obese diabetic mice (*db/db*) were treated with a low fat standard diet (LF), and high fat diets enriched with saturated and monounsaturated fatty acids (lard oil; HF/S), n-6 PUFA (safflower oil) and the latter replacing 40% of fatty acids by marine n-3 PUFA for 6 weeks. Gene expression from gonadal and subcutaneous white adipose tissue was analyzed by oligonucleotide microarrays and quantitative real-time RT-PCR.
Results: HF/S treated *db/db* mice dramatically induced expression of MMP-12 and other genes involved in matrix degradation including MMP-3, -14, a disintegrin-like and metalloproteinase (ADAM)-8 and cathepsin K in gonadal and subcutaneous adipose tissue compared to mice on LF diet. Histological sections revealed an increase in mean adipocyte area by 1.6-fold in HF/S treated mice compared to mice on the LF diet. Immunofluorescence staining of MMP-12 showed co-localization with macrophages but also with adipocytes. Genes essential for matrix collagen production and non-collagenous glycoproteins such as procollagen I, III, VI, arginase I, tenascin C and biglycan were upregulated in mice treated with HF/S. Inclusion of n-3 PUFA completely blunted the HF/S-induced upregulation of genes involved in matrix degradation and production and restored mean adipocyte area compared with LF treated mice. In addition, MMP-12 protein deposition in macrophages and adipocytes was prevented by n-3 PUFA.
Conclusion: N-3 PUFA prevent matrix remodeling and adipocyte enlargement in white adipose tissue of obese diabetic mice. These changes could contribute to the improvement of insulin sensitivity.
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A diet high in saturated fat blunts the tissue-specific adaptation of lipoprotein lipase activity to feeding in rats

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Background and Aims: Accumulation of lipid within skeletal muscle, rather than peripheral fat depots, has been associated with the development of the Metabolic Syndrome. A key determinant of inter-tissue partitioning of circulating lipids is lipoprotein lipase (LPL) which hydrolyses circulating triglyceride (TG) to free fatty acids (FA) to allow their uptake by tissues. Here we have employed a novel tracer technique to investigate *in vivo* the effect of diet on tissue-specific activity of LPL. Our aim was to determine whether a high-fat diet, previously shown to modulate the metabolism of free FA, also affected the metabolism of tryglyceride-derived FA.
Materials and Methods: Male Wistar rats were maintained on a diet of standard laboratory chow (LO-FAT) or an isocaloric diet high in saturated fat (HI-FAT). A lipid emulsion containing ^{14}C-tripalmitin radiolabelled VLDL-like particles, together with ^{3}H-palmitate tracer bound to albumin was administered *iv* to anaesthetised rats. Clearance terms, K_{TG} and K_{FA}, were calculated from the specific activity in plasma and the tissue accumulation of each radiolabel in storage products of individual tissues. Since TG-derived FA and free FA share common metabolic pathways after TG hydrolysis, within each tissue, the ratio K_{TG}/K_{FA} is an index of lipase activity.

Results:

LIPASE ACTIVITY (K_{TG}/K_{FA}x10^{-2})

TISSUE	LO-FAT FASTED	LO-FAT FED	HI-FAT FASTED	HI-FAT FED
Red Muscle	4.2 ± 0.6	2.1 ± 0.4*	2.7 ± 0.1§	3.1 ± 0.4
White adipose tissue	3.7 ± 0.3	27.2 ± 4.0*	3.3 ± 0.2	15.3 ± 2.2*§

*P<0.002 FED *vs* FASTED §P<0.01 HI-FAT *vs* LO-FAT

Conclusion: We conclude that the fasting-fed transition in LO-FAT rats results in a decrease in muscle lipase activity, and an increase in white adipose tissue (WAT) lipase activity, which would promote partitioning of FA derived from dietary TG towards WAT and protect muscle from lipid oversupply. The feeding response is blunted in WAT, and absent in muscle, of rats fed a high-saturated-fat diet. We suggest this dietary adaptation is a significant contributing factor to the muscle lipid accumulation and insulin resistance associated with a high-fat intake.

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Exercise training mediated up-regulation of PPARδ improves insulin resistance in high fat diet induced obese rat

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Background and Aims: Exercise training is believed to improve insulin resistance(IR), however, it is still not quite known how exercise training ameliorates IR. The peroxisome proliferator-activated receptors (PPARs) are dietary lipid sensors that regulate fatty acid and carbohydrate metabolism. Recent research has demonstrated that PPARδ, a member of PPARs family, widely spread, especially rich in muscle, is important in regulating lipid metabolism, enhancing lipid catabolism and fat burning. In this study, we investigate the effect of exercise training on insulin resistance, serum adipose cytokines release and the expression of PPARδgene mRNA of muscle and adipose tissue in high fat diet induced obese rat.
Materials and Methods: The male Wistar rats were divided randomly into 4 groups (each group containing at least 15 rats): regular diet (RD), high fat diet (HFD), high fat diet simultaneously with swimming exercise for 12 weeks(E-HFD) and swimming training for 6 weeks after high fat diet for 6 weeks(HFD-E). Insulin sensitivity index(ISI, ISI=ln[1000/(FPG×FINS)]), serum free fatty acid (FFA), leptin, TNF-α and IL-1 were detected before and after exercise training. The expression of PPARδ gene in muscle and adipose tissue were measured with real-time PCR technique after exercise training.
Results: Compared with HFD, exercise training increased ISI in both E-HFD (2.09 ± 0.32 vs 1.13 ± 0.21, p<0.05) and HFD-E group (1.80 ± 0.34 vs 1.13 ± 0.21,p<0.05), and decreased the serum adipokines level significantly in E-HFD group (FFA: 0.32 ± 0.13 vs 0.57 ± 0.16 mmol/L, leptin: 1.59 ± 0.47 vs 2.85 ± 0.92 ng/ml, IL-1: 33.46 ± 12.25 vs 72.14 ± 13.65 pg/ml TNF-α: 81.03 ± 15.65 vs 101.32 ± 23.79 pg/ml, p<0.05) and HFD-E group (FFA: 0.41 ± 0.13 vs 0.57 ± 0.16 mmol/L, leptin: 1.62 ± 0.77 vs 2.85 ± 0.92 ng/ml, IL-1: 45.78 ± 10.39 vs 72.14 ± 13.65 pg/ml TNF-α: 92.03 ± 18.82 vs 101.32 ± 23.79 pg/ml, p<0.05). Consistently, exercise increased PPARδgene expression in quadriceps and perinephric fat in E-HFD (quadriceps: 630 ± 58 vs 129 ± 30% fat: 473 ± 72 vs 156 ± 42%, p<0.01) and HFD-E (quadriceps: 481 ± 53 vs 129 ± 30%, fat: 340 ± 39 vs156 ± 42%, p<0.01).
Conclusion: The mechanism of exercise training ameliorating IR is at least partly through up-regulation of PPARδ gene, enhancement of fatty acids oxidation, reduction of serum adipose cytokines level and thus amelioration of IR.

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High-fat diet decreased the expression of AMPK-alpha of rats' skeletal muscle

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Background and Aims: High-fat diet is a factor leading to insulin resistance, while the mechanisms are not fully elucidated. Several studies support the hypothesis that AMPK plays an important role in the stimulation of muscle glucose uptake by physiological and pharmacological stimuli. But whether AMPKa1 or a2 subunit (the different isoforms of AMPK catalytic subunit) have a dominant role in muscle glucose uptake is controversial. Here we investigated the effect of AMPKa in chronic high-fat diet on insulin resistance**.**
Materials and Methods: The animal study was approved by the Shandong University Institutional Animal Care and Use Committee. Male Wistar rats were randomly divided into three groups: control group (320 kJ · day^{-1}; standard diet), high-fat diet group (320 kJ · day^{-1}; high fat diet: 59% fat, 20% carbohydrate, and 21% protein) and metformin-treated group (320 kJ · day^{-1}; high fat diet). Metformin (activator of AMPK) was administrated orally with the dose of 50 mg· kg^{-1} · d^{-1}. Feeding for 20 weeks, fasting and glucose-loaded 2 h blood glucose levels were measured by the glucose-oxidase method. Rats were anesthetized and the gastrocnemius muscles were dissected out rapidly. Some tested for the ability of insulin-stimulated glucose uptake of rats' skeletal muscle in-vitro; Other muscles were frozen for the following experiments. mRNA levels of Glut4, AMPKa1 subunit and AMPKa2 subunit were determined using Real-time PCR. Protein levels of total AMPKa subunit and P-AMPKa (the activity form of AMPKa) subunit were measured using Western blot.
Results: (1) Compared with control, high-fat diet elevated both fasting and glucose-loaded 2 h blood glucose levels (p<0.05) accompanied with both decreased basal (p<0.05) and insulin-stimulated(p<0.01) glucose uptake. (2) High-fat diet feeding impaired the protein expression of AMPKa (p<0.05), especially shew deeply down-regulation in protein level of P-AMPKa (p<0.05). (3) High fat decreased mRNA level of AMPKa2 subunit (p<0.05), not AMPKa1 subunit. (4) mRNA level of GluT4 decreased after high-fat diet feeding in accord with the alteration of P-AMPKa and muscle glucose uptake. (5) Compared with HF group, administration of metformin could increase the protein levels of both AMPKa (p<0.05) and P-AMPKa (p<0.01), and ameliorating GluT4 expression (p<0.01).
Conclusion: High-fat diet impaired the mRNA and protein expression and activity of AMPKa of rats' skeletal muscle, implying more important role of AMPKa2 in the lipotoxicity.

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Increased myocardial expression of PGC1-target genes in high-fat diet fed rats

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Background and Aims: A shift in cardiac substrate metabolism, i.e. an increase in fatty acid oxidation (FAO) at the expense of glucose oxidation, leading to altered energy metabolism, may contribute to the development of diabetic cardiomyopathy (DCM). Peroxisome proliferator-activated receptor γ coactivator-1 (PGC1) is a key regulator of cardiac energy metabolism and mitochondrial function. We examined whether exposure of rats to excessive alimentary fat, thereby inducing a type 2 diabetic phenotype and cardiac functional changes resembling DCM, alters the expression of genes determining mitochondrial function and cardiac energy metabolism.

Materials and Methods: Male Wistar rats received isocaloric high (HFD) and low-fat (LFD) diets for 7 weeks. Gene and protein expression were assessed in cardiac ventricular lysates using real-time PCR and Western blotting.
Results: Mean heart weight, but not body weight, was increased in HFD-rats ($P<0.05$). HFD-hearts showed triglyceride accumulation and ultrastructural abnormalities indicating mitochondrial degeneration. Mitochondrial copy number was slightly elevated in HFD-fed hearts (1.5-fold, P=0.242), while protein expression of manganese superoxide dismutase, an indicator of oxidative stress, and of cytochrome c oxidase subunit 1 were significantly increased in HFD- vs LFD-hearts (both $P<0.05$). Although, the expression of PGC1α- and PGC1β- was not significantly increased in HFD-hearts, the PGC1-regulated genes MCAD, CPT1α and CPT1β were elevated in HFD- vs LFD-hearts (all $P<0.05$). Also, gene expression of the PGC1-regulated transcription factors PPARα, PPARδ, and NRF1 were increased in HF-hearts (all P<0.05). Finally, HFD-hearts showed a 3.4-fold increase in pyruvate dehydrogenase kinase 4 protein levels and acetylCoA carboxylase-phosphorylation (all P<0.05).
Conclusion: Exposure to HF-diet induces changes in cardiac gene expression and protein levels suggestive of an increased FAO, mitochondrial dysfunction and oxidative stress. Collectively, these alterations may contribute to the development of diabetes-related heart disease.

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Hepatic overexpression of plasma membrane-associated sialidase (NEU3) by adenoviral transduction improves insulin sensitivity and glucose tolerance in mice fed a high-fat diet and KKAy mice
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Background and Aims: Plasma membrane-associated sialidase, NEU3, is a key enzyme for ganglioside hydrolysis. Membrane microdomains rich in ganglioside are now recognized as critical for proper compartmentalization of insulin signaling. We generated mice overexpressing Neu3 and reported that the mice developed diabetic phenotype associated with hyperinsulinemia, islet hyperplasia, and increased beta-cell mass. In this study, we investigated the effects of hepatic NEU3 overexpression on in vivo insulin sensitivity in C57BL/6N mice fed normal and high-fat diets, as well as KKAy mice.
Materials and Methods: Adenoviral-mediated gene transfer was used to overexpress NEU3 in the livers of male mice. The active adenovirus (AdNEU3) was constructed using the AdEasy system (Stratagene) and contained the human NEU3 cDNA under control of CMV promoters. An adenovirus containing LacZ cDNA (AdLacZ) was used as a control. Male C57BL/6N mice were given free access to a high-fat diet for 6 weeks. The C57BL/6N mice and KKAy mice received a single dose of AdNEU3 or AdLacZ adenoviruses by tail-vein injection, resulting in liver-specific infection. Glucose tolerance and insulin sensitivity were assessed by intraperitoneal glucose tolerance test and intravenous insulin tolerance test.
Results: Compared with the controls, the AdNEU3-mice had significantly >5-fold higher mRNA expression, protein contents and enzymatic activities of NEU3 in liver at 5–7 days post-infection, but no changes in other tissues such as skeletal muscle and adipose tissues. NEU3 overexpression in liver significantly improves glucose tolerance and insulin sensitivity in the C57BL/6 mice fed normal and high-fat diets, as well as KKAy mice. AdNEU3-mice had significantly higher glycogen deposition in liver. NEU3 overexpression also promoted triglyceride deposition and increased the expression of PPARgamma in liver of C57BL/6N mice with normal and high-fat diets, as well as KKAy mice. HTLC analysis demonstrated increased levels of GM1 and GM2, in contrast, marked reduction in GM3, a negative regulator of insulin signaling, in the liver of NEU3ad mice. Thus, insulin-stimulated tyrosine phosphorylation of insulin receptor substrate 1 was significantly increased, but no changes in insulin receptor (IR) tyrosine phosphorylation in the liver of NEU3ad mice. Insulin-stimulated IR tyrosine phosphorylation was increased in adipose tissues of NEU3ad mice.
Conclusion: These results suggest that NEU3 overexpression in liver improves insulin sensitivity and glucose tolerance through the modification of ganglioside composition in normal and two types of insulin-resistant mice, high-fat diet and KKAy mice. Our findings also provide further evidence that ganglioside in membrane microdomains is an important regulator of insulin signaling, making it a potential therapeutic target in type 2 diabetes.

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Effects of an inhibitor of 11beta-hydroxysteroid dehydrogenase type 1 inhibitor on energy balance and glucose homeostasis in diet-induced obesity
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Background and Aims: Inhibition of 11β-hydroxysteroid dehydrogenase type 1 (11βHSD-1) reduces the conversion of inactive (cortisone to cortisol in humans; dehydrocorticosterone to corticosterone in rodents) to active glucocorticoids in tissues. Support for this approach in the treatment of the metabolic syndrome has come from studies on the selective 11βHSD-1 inhibitor BVT.2733 in various mutant mouse models of obesity and diabetes. Food intake and body weight were reduced in some experiments, and glucose and insulin levels were reduced even in experiments in which no effect on energy balanced was detected. We have investigated whether and how BVT.2733 reduces body weight and improves glucose tolerance in diet-induced obese mice.
Materials and Methods: Female C57Bl/6 mice (aged 12 months) that had been made obese by feeding on a high fat diet were housed in 3 or 4 groups of 3 and dosed twice daily with vehicle or BVT.2733 (100 mg/kg body weight, p.o.) for 16 or 17 days. One group was pair-fed to the BVT.2733 group. Effects described are $p < 0.05$ (one-way ANOVA and Dunnett's or Bonferroni's multiple comparison test; n = 3 or 4 for food and water intake and energy expenditure; 9 -12 for other measurements).
Results: BVT.2733 reduced food intake by 26 ± 6% and body weight gain (–3.9 ± 1.2 vs. 0.5 ± 1.0g), but increased water intake by 66 ± 13%. Pair feeding caused almost as great a decrease in body weight (–2.7 ± 0.7g) as BVT.2733. Energy expenditure over 22 h on days 11 to 12 was reduced by 21 ± 2% in the pair-fed mice. It was higher in the BVT.2733-treated mice than the pair-fed mice by 38 ± 8%. At termination both fat and lean (dual-energy X-ray absorptiometry) were reduced in the pair-fed mice and percentage fat was unchanged (control, 47.8 ± 2.6; pair-fed, 47.1 ± 1.9%), whereas BVT.2733 did not reduce lean and reduced percentage fat (40.9 ± 2.0%). BVT.2733 was less effective than pair-feeding in reducing fasting blood glucose (days 15, 16), and BVT.2733 (12 ± 3% reduction) but not pair-feeding (4 ± 4%) reduced both the total area under the glucose (1.5g/kg, i.p.) tolerance curve and the plasma insulin concentration 30 min after giving glucose (BVT, by 40 ± 8%; pair-feeding, by 16 ± 10%).
Conclusion: BVT.2733 prevented diet-induced obesity both by decreasing food intake and preventing decreased energy expenditure. Improvement of glucose homeostasis was partly secondary to decreased food intake; thermogenesis may also have played a role. Stimulation of water intake may have been due to diuresis and requires further investigation.

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Fenofibrate treatment improves insulin sensitivity in mice with diet-induced obesity despite paradoxical increase in circulating resistin levels
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Background and Aims: Obesity-induced insulin resistance is accompanied by significant changes in endocrine function of adipose tissue that contribute to the overall metabolic phenotype. The aim of this study was to explore the significance of adipose tissue-derived hormones resistin and adiponectin in the development of insulin resistance induced by lipogenic, simple carbohydrate diet and in the insulin-sensitizing effects of PPAR-alpha activation.
Materials and Methods: Male C57BL/6J mice were fed either normal chow or lipogenic, simple carbohydrate diet (AIN-93G, Dyets Inc., USA) for 12 weeks. Mice on both diets were then treated with PPAR-alpha agonist fenofibrate (100 mg/kg) administered in the food for 14 days. Untreated mice on both diets served as controls. Biochemical and hormonal parameters were measured using commercial RIA and ELISA kits and insulin sensitivity was assessed by euglycaemic-hyperinsulinemic clamp using [3-^{3}H]glucose and 2-deoxy-D-[1-^{14}C] as tracers.
Results: Lipogenic diet (LD) feeding significantly increased body weight, gonadal fat pad weight and insulin levels relative to chow-fed group (34.3 ± 0.8 vs. 27.6 ± 0.9 g; 1.14 ± 0.23 vs. 0.27 ± 0.01 g, 0.81 ± 0.08 vs. 0.36 ± 0.1 ng/ml, $p<0.05$) Fenofibrate treatment decreased body weight and fat pad weight in both chow-fed and LD-fed mice with concomitant reduction in blood glucose, free fatty acid, triglyceride and serum insulin levels. Euglycaemic-hyperinsulinemic clamp demonstrated the development of

both whole body and liver insulin resistance in LD-fed mice which was both normalized by fenofibrate treatment (whole body glucose uptake in LD: 203 ± 11 μmol/kg/min vs. 328 ± 21 μmol/kg/min in chow-fed mice vs. 354 ± 26 μmol/kg/min in LD+fenofibrate-fed mice, $p<0.05$). Resistin levels tended to be lower in LD-fed mice relative to chow-fed group (12.6 ± 0.7 ng/ml vs. 20.3 ± 2.9 ng/ml, $p<0.05$) and were two-fold increased by fenofibrate treatment on both LD and chow-fed mice (24.9 ± 4.2 ng/ml and 36.3 ± 2.6 ng/ml, respectively). Adiponectin levels were not affected by LD feeding and increased after fenofibrate treatment in chow-fed but not LD-fed mice.
Conclusion: Changes in adiponectin and resistin levels were not involved in LD feeding-induced insulin resistance. Fenofibrate treatment prevented development of obesity and improved insulin sensitivity in LD-fed mice despite major increase in serum resistin levels suggesting that resistin may not be the major factor in the development of liver insulin resistance in mice. We conclude that changes in adiponectin or resistin systemic levels are not responsible for insulin-sensitizing effects of PPAR-alpha activation.
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Insulin signalling and glucose transport

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Initial phosphorylation of insulin receptor substrate 1 at Ser-318 positively stimulates insulin signal transduction and is a prerequisite for sequential inhibitory steps
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Background and Aims: The important physiological balance between activation and termination of insulin action can be modulated by Ser-/Thr-phosphorylation of insulin receptor substrate (IRS)-1, which is one of the key elements in the stimulation and attenuation of insulin signal transduction. Therefore, we aimed to investigate the regulation and function of Ser318 phosphorylation (pSer318) of IRS-1 in skeletal muscle, as a major organ for insulin-stimulated glucose disposal in vivo.
Materials and Methods: We detected the phosphorylation of Ser318 in cellular extracts and tissues using a recently prepared polyclonal site-specific antibody by Western blotting. The effects on the insulin signaling were investigated by Western blotting. The biological function of pSer318 have been studied using a Ser318 to Ala or Glu mutants of IRS-1.
Results: We show that in muscle tissue of mice and in skeletal muscle cell models Ser318 is rapidly phosphorylated by insulin (< 5 min) and that this phosphorylation is mediated by protein kinase C (PKC)-ζ. The in vivo phosphorylation of Ser318 is further reflected by induction of Ser318 phosphorylation in lymphocytes of these insulin-treated mice and in human lymphocytes obtained after an euglycemic-hyperinsulinemic clamp. IGF-1, but not pathological stimuli like TNF-α or LPS, also induced Ser318 phosphorylation. The dissociation of the insulin-induced PKC-ζ and IRS-1 complex after 10 min was probably caused by Ser318 phosphorylation, since we found no PKC-ζ interaction with Glu318 IRS-1, which simulates a permanently phosphorylated state.
Replacing Ser318 with alanine reduces short-term insulin-stimulated Akt phosphorylation but prevents the reduction of insulin-mediated Akt phosphorylation after chronic insulin stimulation. Moreover, introduction of Ala318 led to accumulated glucose uptake after long-term (60 min) insulin stimulation. In the early phase of insulin action the stimulating function of the phosphorylation of Ser318 on glucose transport is demonstrated by replacing Ser318 with glutamate, which enhances glucose uptake after 5 and 20 min of insulin stimulation.
Conclusion: We conclude that insulin-stimulated Ser318 phosphorylation of IRS-1 is an physiological event which probably supports insulin signal transduction in the early phase but is required for the sequential attenuation of the ongoing signaling cascade.

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Direct cross-talk of interleukin-6 (IL-6) and insulin signal transduction via insulin receptor substrate (IRS)-1 in skeletal muscle cells
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Background and Aims: The exercise-induced IL-6 production and secretion within skeletal muscle fibers has raised the question of a putative function of IL-6 in the energy metabolism of the muscle during and after the exercise. In the present study we followed the hypothesis that IL-6 may directly interact with important molecules in the insulin signaling cascade.
Materials and Methods: Co-immunoprecipitation of IL-6 receptor complex and insulin signaling molecules was studied in C2C12 cells, IL-6-induced Ser-318 phosphorylation of IRS-1 was monitored in various muscle cells and in vivo, glucose uptake was studied in L6 myotubes.
Results: We show that IL-6 induces a rapid recruitment of two keystones in insulin signal transduction, insulin receptor substrate (IRS)-1 and the p85 subunit of PI-3 kinase, to the IL-6 receptor complex in C2C12 cells. The association of p85 is not mediated by tyrosine phosphorylation of IRS-1, since IL-6 did not induce this modification of IRS-1. Moreover, IL-6 induces a rapid and transient phosphorylation of Ser-318 of IRS-1 in cultured skeletal muscle cells and in muscle, but not in the liver of IL-6 treated mice, probably via the IL-6-induced co-recruitment of PKC-δ. Overexpression of PKC-δ, but not of kinase negative PKC-δ, resulted in a strong phosphoryla-

tion of Ser-318 also in unstimulated cells. This Ser-318 phosphorylation improves insulin-stimulated glucose uptake in L6 myotubes, stably transfected with a IRS-1/Glu-318 mutant to simulate a stable phospho-Ser-318 modification.
Conclusion: The data provide evidence for a possible molecular mechanism of the metabolic effects of IL-6 in skeletal muscle, thereby exerting short-term beneficial effects on insulin action. This possibly physiological function of exercise-induced IL-6 has to be discriminated from the pathophysiological function of chronically elevated circulating levels of IL-6 which are able to induce insulin resistance.

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Insulin sensitization on glucose uptake in myoblasts null for the protein phosphatase PTP1B
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Background and aims: PTP1B is a negative regulator of insulin signaling and a therapeutic target for type 2 diabetes. Mice lacking PTP1B exhibit enhanced insulin sensitivity attributable in part to increased IR phosphorylation in muscle. This tissue accounts for the 80% of the insulin-stimulated glucose uptake and is the organ where insulin resistance is primarily detected. Accordingly, the purpose of the present study was to investigate the mechanism by which this phosphatase regulates insulin sensitivity in immortalized myoblasts PTP1B-null.
Materials and Methods: PTP1B -/-, -/+ and +/+ myoblasts were obtained by immortalization with retroviral construct of SV40 Large T Ag of primary mouse neonatal myoblasts isolated from wild type or null 129sv PTP1B mice. The genotype of PTP1B-/- cells was checked by PCR. Phenotypic characterization confirmed the absence of PTP1B and the expression of muscle specific genes.
Results: PTP1B-/- myoblasts showed a higher IR Tyr phosphorylation than +/+ cells, with stimulation at lower doses of insulin and after shorter-time of incubation. This time and dose increased sensitivity to insulin was also detectable on Tyr phosphorylation of IRS-1 and IRS-2, PI3K-associated activation and Ser/Thr phosphorylation of AKT. However, no changes were observed at the level of PKCzeta. In consequence, both insulin-stimulated glucose uptake and GLUT4 translocation to the plasma membrane were increased in PTP1B null cells compare with wild type cells.
Conclusions: Lack of PTP1B in muscle cells increased insulin sensitivity and glucose uptake and could confer protection against insulin resistance under determined environmental conditions.
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Functional role of caveolae in insulin-induced activation of phosphodiesterase 3B in primary adipocytes
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Background and Aims: Caveolae are 25–150 nm invaginations of the plasma membrane which are particularly abundant in rat adipocytes and which may be important in organizing insulin signalling and lipid homeostasis. A number of proteins involved in signal transduction have been found in this structure and disruption of caveolar integrity by cholesterol depletion using methyl-β-cyclodextrin reversibly makes the cells insulin resistant by inhibiting, for example, the insulin receptor from phosphorylating insulin receptor substrate-1. Thus, caveolae has to be taken into account when trying to understand normal insulin signal transduction as well as the dysfunction that causes insulin resistance. In caveolin-1 knock-out mice which lack caveolae one can see a reduced amount of white adipose tissue, increased adipose tissue protein kinase A (PKA) activity and no reduce in serum free fatty acids after feeding compared to control mice, indicating increased adipocyte lipolysis/lack of the antilipolytic action of insulin. One important metabolic action of insulin is to inhibit catecholamine-induced lipolysis, mainly mediated via insulin-induced activation and phosphorylation of phosphodiesterase 3B (PDE3B). Activation of PDE3B results in increased hydrolysis of cAMP, decreased activity of PKA and thereby decreased cAMP/PKA mediated activity of hormone sensitive lipase. The role of caveolae in the regulation of PDE3B and thereby lipolysis is not known, hence the aim of this study is to further examine this connection.
Materials and Methods: Adipocytes were prepared from epididymal adipose tissue of 38–42 day-old male Sprague-Dawley rats and incubated with different stimuli before homogenisation. The homogenates were analyzed using different methodologies: subcellular fractionation, gelfiltration chromatography, detergent solubilization, sucrose gradient centrifugation, immunoprecipitation, SDS-PAGE, western blotting, cholesterol measurement and PDE activity/protein determinations depending on the aim of the experiment.
Results: In this study we show that PDE3B from primary rat adipocytes to a large extent is present in detergent-insoluble regions of the plasma membrane such as caveolae or lipid rafts. Enrichment of caveolae using sucrose density gradient centrifugation or immunoprecipitations with caveolin-1 antibodies demonstrates co-localization between caveolae/caveolin-1 and PDE3B. In addition gelfiltration chromatography of membrane fractions from primary adipocytes show that PDE3B elutes together with cholesterol, caveolin-1 and flotillin-1 at a molecular weight (Mw) larger than 5 000 kDa. Stimulation of adipocytes with insulin results in an increased amount of PDE3B activity in the high Mw peak and pre-treatment of the cells with methyl-β-cyclodextrin results in a disappearance of the high Mw PDE3B peak.
Conclusion: In summary, these results indicate an association of PDE3B with caveolae and a functional role for this association in insulin-induced activation of PDE3B. Thus, our data support previous results on an important role of caveolae in organizing insulin signalling and also underline that dysfunction of caveolae should be taken into consideration in development of insulin resistance.

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Analysis of insulin resistance in 3T3-L1 adipocytes using specific kinase inhibitors
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Background and Aims: Insulin signaling can be negatively regulated by phosphorylation of the insulin receptor substrate (IRS)-1 on serine residues. This modification has been proposed to play a key role both in feedback inhibition of the insulin signaling cascade and in the development of cellular insulin resistance. However, the liable serine kinases and underlying mechanisms remain to be determined. The aim of the present study was to characterise the mechanisms of experimentally induced insulin resistance in 3T3-L1 adipocytes using highly specific kinase inhibitors.
Materials and Methods: 3T3-L1 adipocytes were exposed over night to TNFα (20 ng/ml) or high glucose (25 mmol/l) in the presence of insulin (10 nmol/l). After insulin stimulation, the effect of kinase inhibitors for mTOR, JNK, GSK3β, IKK, PKC or MEK1 and aspirin were studied with regard to phosphorylation of signaling molecules, IRS-1 and Akt protein levels and downstream metabolic effects.
Results: Sustained incubation with TNFα or high glucose plus insulin resulted in reduction of insulin-stimulated Akt phosphorylation (48 ± 13% and 41 ± 6% respectively) and decreased glucose uptake (66 ± 6% and 53 ± 7% respectively). The observed effect of TNFα was primarily due to a prominent reduction of IRS-1 and Akt protein levels. Further on the stress kinase JNK was activated just transiently with a maximum 10 minutes after addition of TNFα. Under these conditions only the treatment with a high dose of aspirin (5 mmol/l) could ameliorate the acute insulin-stimulated glucose transport. High glucose plus insulin did not affect the protein levels of signaling proteins, but resulted in a marked reduction in the fold-stimulation of tyrosine phosphorylation of IRS-1, which may be responsible for the observed reduced signal transduction. Interestingly, neither the treatment with highly specific kinase inhibitors for JNK, GSK3β, IKK, PKC or MEK1, nor the analysis of serine phosphorylation of IRS-1 argued for an involvement of these kinases and therefore inhibitory serine phosphorylation. Only the treatment with rapamycin for one hour resulted in normalisation of insulin-induced Akt phosphorylation and glucose uptake in a dose dependent manner.
Conclusion: Treatment of 3T3-L1 adipocytes either with TNFα or high glucose plus insulin is widely used for the induction of insulin resistance. Our results indicate that under both conditions the activity of the serine kinases JNK, GSK3β, IKK, PKC or MEK1, which have been described to be involved in the pathophysiology of type 2 diabetes, are not responsible for the observed impairment of insulin signaling and glucose uptake in this cellular model. These results indicate also that mTOR or downstream effectors of the mTOR pathway are important negative modulators of the insulin signaling cascade in 3T3-L1 adipocytes.

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Heat shock protein 90 is involved in the development of muscle insulin resistance

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Background and Aims: Heat shock protein 90 (Hsp90) is a chaperone that buffers/modulates insulin signalling at the level of protein kinase B (Akt) phosphorylation. Interaction with Hsp90 stabilizes Akt protein, but it is also able to dampen insulin signalling by enhancing Akt dephosphorylation. The potential contribution of this process to insulin resistance remains unknown. The aim of this study was to explore the effect of Hsp90 inhibition under conditions of impaired insulin signalling in a muscle cell line.
Materials and Methods: E2-cardiomyocytes were treated with 100 nM insulin and 20.5 mM glucose overnight to induce insulin resistance. After washing followed by 1 h serum free incubation, the cells were stimulated for 5' with 100 nM insulin. Activation of the signalling cascade was assessed by measuring the phosphorylation of Akt. Inhibition of Hsp90 was induced by geldanamycin.
Results: Incubation with high concentrations of glucose and insulin led to an additive reduction of Akt phosphorylation by up to 60% when compared to untreated control. Glucose/insulin treatment did not affect the expression of the insulin receptor, Hsp90 and Akt, and did not modify autophosphorylation of the receptor. Inhibition of IKK, mTOR, GSK3 and JNK by highly specific compounds was unable to prevent impaired insulin signalling by glucose/insulin treatment. Further, activation of JNK by anisomycin did not impair Akt signalling. Inhibition of Hsp90 by geldanamycin doubled the level of Akt-phosphorylation in the glucose/insulin treated cells and diminished the inducible resistance from 60% to about 20% when compared with the untreated control. The dephosphorylation rate of Akt was estimated by inhibiting PI3-kinase and measuring the decrease in Akt phosphorylation. Incubation with geldanamycin blocked the dephosphorylation of Akt. However, there was no difference in the dephosphorylation rate between glucose/insulin treated and untreated cells.
Conclusion: Inhibition of Hsp90 almost completely restored the glucose/insulin induced impairment of insulin signalling at the level of Akt kinase. The serine kinases IKK, JNK, GSK3 and mTOR were not involved in the development of this signalling deficiency. Since the dephosphorylation rate of Akt remained unchanged, the phosphatase activity itself seems to be unaffected. Therefore, we propose that the Hsp90 chaperone plays an important role in insulin resistance by mediating an increased exposition of Akt to the dephosphorylation process.

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The role of protein kinase C delta and signal transducer and activator of transcription 3 in insulin induced cell proliferation

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Background and Aims: Transcriptional activation of STAT3 is regulated by STAT tyrosine and serine phosphorylation modulated by cytokines and growth factors including insulin. We have previously shown that primary keratinocytes express functional IR and IGFR and that insulin and IGF-1 regulate keratinocyte proliferation and differentiation. Furthermore, we have identified an insulin signaling pathway which is specifically regulated by PKCδ activation to induce cell proliferation.
Materials and Methods: In the present study we have utilized primary skin keratinocytes and recombinant adenoviruses of WT-PKCδ, kinase inactive PKCδ mutant, STAT3 and STAT3 mutants to examine the crosstalk between PKCδ and STAT3 activation in insulin mediated cell proliferation.
Results: We found that insulin stimulation induced PKCδ-STAT3 association concomitant with STAT3 tyrosine and serine phosphorylation within the STAT3-PKCδ complex. In addition, in response to insulin stimulation, STAT3 specifically interacted with IR but not with the IGF-1 receptor. PKCδ activation mediated STAT3-IR complex formation and STAT3 tyrosine phosphorylation. Specifically, STAT3 was physically associated with PKCδ but not with any other PKC isoform expressed in skin. Furthermore, activated forms of PKCδ and STAT3 were essential for insulin induced PKCδ-STAT3 activation in keratinocyte proliferation. Abrogation of PKCδ activity inhibited insulin-induced PKCδ-STAT3 complex formation, STAT3 activation and nuclear translocation. Furthermore, overexpression of a STAT3 tyrosine mutant eliminated insulin induced PKCδ activation and keratinocyte proliferation. Finally, in order to specifically investigate the role of STAT3 serine phosphorylation in insulin signaling and cell proliferation we have constructed a recombinant STAT3 serine 727 mutant adenovirus. Overexpressing STAT3 serine mutant in keratinocytes abrogated PKCδ mediated STAT3 serine phosphorylation and keratinocyte proliferation. However, STAT3 tyrosine phosphorylation was dramatically induced and nuclear localization was intact.
Conclusion: This study suggests a role for PKCδ in regulation of STAT3 activation and insulin-mediated proliferation. Defective regulation of this pathway as it appears in diabetes patients, suggests a role for insulin mediated PKCδ and STAT3 activation in the pathophysiology of diabetes.

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Insulin induced actin remodelling and GLUT4 translocation in L6 muscle is regulated by the small GTPase Rac

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In muscle and fat, glucose disposal is mediated by translocation of the glucose transporter GLUT4 to the plasma membrane. This depends on a signalling cascade downstream of the insulin receptor, as well as their proper localization, assisted by a remodelling of the actin cytoskeleton. Although the signalling components responsible for GLUT4 translocation and glucose disposal are well known, the effectors in insulin-induced actin remodelling remain to be identified. Here, we hypothesize that insulin-induced actin remodelling in L6 muscle cells is regulated by the small GTPase Rac. We further hypothesize the effectors downstream of Rac include p21-activated kinase (Pak1) and WAVE2.
To determine if Rac mediates insulin-induced actin remodelling we used dominant-inhibitory cDNA mutants of Rac1 and demonstrated an inhibition of lamellipodia formation in L6 myoblasts. Furthermore, knockdown of Rac1 expression in differentiated myotubes accomplished with siRNA, prevented insulin-induced Rac1 activation, actin remodelling and GLUT4 translocation.
To confirm the role of Pak1 in insulin signalling, we determined its activation by immunoblotting for phospho-specific Pak1 at sites S423 and S199. Using cDNA mutants of Pak1, we demonstrated a slight inhibition of insulin-induced actin remodelling when L6 myoblasts over-express kinase-dead or autoinhibitory mutants of Pak1. However, knockdown of Pak1 expression using siRNA did not affect insulin stimulated GLUT4 translocation. Preliminary results with inactive mutants of a WAVE2- mediated Rac activator inhibit insulin stimulated actin remodelling.
Collectively, these observations indicate that the small GTPase Rac1 is responsible for insulin-induced actin remodelling. We propose that, in muscle cells, insulin engages the PI3K to Rac axis in order to remodel actin and facilitate GLUT4 translocation through WAVE2.

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Evaluation of glucose transport and glucotransporter 4 (GLUT4) expression in natural killer (NK) cells of healthy subjects and type 2 diabetic patients

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Background and Aims: Patients with Type 2 diabetes are known to be at high risk for immune disturbances. NK cells are thought to represent the first line defense in the immune system since they kill abnormal cells and simultaneously secrete cytokines to activate the other arms of the immune response. Studies of the glucose transport activity of these cells may contribute to the better understanding of the pathogenetic phenomenon in the natural history of Type 2 diabetes. Therefore the aim of this study was to determine the quantitative expression of insulin-dependent GLUT4 in human NK-cells, which has not been proven so far, and to evaluate if there was a change of glucose uptake in NK cells obtained from diabetics in comparison with healthy subjects.
Materials and Methods: The study group included 6 patients with newly diagnosed Type 2 diabetes, naive to any hypoglycaemic drugs. As a control group 8 carefully matched healthy subjects were enrolled. Circulating lymphocytes from human peripheral blood were obtained from heparinised blood by Ficoll-Isopaque gradient centifugation. NK cells were isolated and removed by anti-CD16 antibody treatment. Cells were stained by using anti-human GLUT4 antibody and FITC-conjugated immunoglobulin. The

expression of GLUT4 was investigated by flow cytometry, which was performed utilizing a FACSCalibur (Becton-Dickinson). Glucose transport was monitored with deoxy-D-glucose (2-[^{3}H(G)]). At previously assigned time points (15, 30, 60 minutes of incubation) deoxy-D-glucose uptake was stopped and its concentration in the cells was measured by scintillation counting.
Results: The results revealed the significant differences between deoxy-D-glucose transport in NK cells obtained from patients with Type 2 diabetes and the control group ($P<0.001$). The mean values of 4564 ccpm at 15 min. 9423 ccpm at 30 min. and 9860 ccpm at 60 min. were obtained in NK cells of diabetics. The glucose uptake was, respectively, 2198 ccpm, 4255 ccpm and 8791 ccpm in NK cells of healthy subjects. The increase of glucose uptake correlated with the level of GLUT4 in the studied cells. Flow cytometry showed the substantial increase in GLUT4 protein level in NK cells from diabetic patients versus control group (95 ± 3,7% vs 14 ± 1,9%). This difference is very significant.
Conclusion: The high expression of GLUT4 in NK cells of Type 2 diabetic patients has been revealed. That may be the cause for a different pattern of glucose transport in these cells in Type 2 diabetes. Such changes in glucotransporter expression may profoundly alter the immune response by influencing NK cell cytotoxicity and cytokine production. Furthermore, NK-cells may be a valid model system to study the cellular glucose transport.
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Melatonin stimulates glucose transport via PI3-kinase pathway in C_2C_{12} murine skeletal muscle cells
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Background and Aims: Glucose uptake in skeletal muscle lies at the center when dealing with diabetes mellitus. Present study was carried out to investigate whether melatonin, a hormone of pineal gland, plays a role in glucose transport in skeletal muscle cells. Although many reports concerning melatonin effect on circadian rhythm have been published, little is known about its effect on glucose transport.
Materials and Methods: We first investigated the effect of melatonin on glucose uptake in C_2C_{12} mouse skeletal muscle cells. To further elucidate underlying mechanism, we studied the activity of phosphatidylinositol 3-kinase (PI3-kinase), another important mediator of insulin dependent glucose transport pathway. Activities of other intermediary mediators such as IRS-1, PKC-zeta, p38, were also investigated. Furthermore, we stably overexpressed melatonin receptor type 2 (MT2) in C_2C_{12} cells to confirm the receptor mediated action of melatonin.
Results: Intriguingly treatment of C_2C_{12} cells with melatonin (10 nM and 100 nM) stimulated glucose uptake up to 2 fold increase. And melatonin stimulated glucose transport was inhibited with co-treatment of melatonin receptor antagonist Luzindole. We also found that melatonin increased the phosphorylation level of insulin response substrate-1 (IRS-1), an important intermediary mediator of insulin dependent glucose transport pathway. We found that the activity of PI3-kinase was increased by melatonin. However, AMP activated protein kinase (AMPK), recently discovered glucose transport stimulating molecule via insulin independent pathway was not influenced by melatonin treatment. Activity of p38 mitogen activated protein kinase (MAPK), downstream mediator of AMPK, was also not changed by melatonin. We observed that glucose transport in MT2 transfected C_2C_{12} cells was amplified up to 15 fold.
Conclusions: In summary melatonin stimulates glucose transport in C_2C_{12} cells via PI3-kinase and IRS-1 pathway.
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GUT- and CNS-mediated effects on metabolism

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Intravenous administration of ghrelin differentially affects hepatic and peripheral insulin sensitivity
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Background and Aims: Ghrelin, secreted mainly by the stomach, has an important role as an orexigenic hormone by exerting effects on the NPY/POMC arcuate circuitry in the hypothalamus. These effects of ghrelin are mediated by the GHS-1a receptor. Besides exerting effects on food intake, we recently showed that arcuate NPY/POMC neurons affect insulin sensitivity as well.
The aim of the present study was to evaluate the effects of intravenous administration of acetylated ghrelin on insulin sensitivity, independent of food intake. In addition, we studied whether these effects are mediated by the GHS-1a receptor, by evaluation of the effects of an agonist of this receptor (GHRP-6) on insulin sensitivity.
Materials and Methods: Male, C57Bl/6 mice were fasted 4 hours prior to the start of the experiment. PBS (control group) or ghrelin (1 µg/hour) (experimental group 1) or GHRP-6 (0.26 µg/hour) (experimental group 2) infusion was started at the beginning of, and continued during the whole experiment. Whole-body and hepatic insulin sensitivity were measured by hyperinsulinemic euglycemic clamp in combination with ^{14}C-glucose infusion.
Results: Under basal conditions, none of the metabolic parameters was affected by ghrelin compared to the control group. GHRP-6 however, decreased endogenous glucose production (33 ± 4 versus 44 ± 9 µmol/kg/hour, $p<0.01$). Under hyperinsulinemic conditions, ghrelin hampered the inhibitory effect of insulin on hepatic glucose production, indicating hepatic insulin resistance (46 ± 22 versus 71 ± 11%, $p<0.05$). In contrast, glucose disposal was higher in ghrelin-treated mice (77 ± 16 versus 59 ± 8 µmol/kg/hour, $p<0.05$). Interestingly, GHRP-6 did not affect hepatic (70 ± 22%) or peripheral insulin sensitivity (60 ± 9 µmol/kg/hour) compared to the control group.
Conclusion: Ghrelin differentially affects tissue-specific insulin sensitivity, i.e. it induces a decrease in hepatic insulin sensitivity but an increase in peripheral insulin sensitivity, independently of its well-known impact on feeding and body weight. These effects of ghrelin do not seem to be mediated via the GHS-R1a.
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Ghrelin inhibits insulin response to glucose infusion into the portal vein through an adrenergic mechanism
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Background and Aims: Ghrelin, a peptide primarily produced in the stomach, regulates both feeding and pancreatic hormone secretion. Secretion of ghrelin is up-regulated under conditions of negative energy balance such as starvation. There seems to be an agreement as insulin is down-regulated by ghrelin. However, there is little knowledge of the pathway conveying the ghrelin's inhibitory effect on the insulin secretion. It has been recognized that insulin secretion after ingestion is enhanced via the vagal neural system. We examined whether ghrelin exerts a neural inhibitory effect on insulin secretion via the portal sensory information, and found that the sympathetic nervous system plays a major role in mediating ghrelin actions on insulin secretion.
Materials and Methods: We used Wistar rats (BW: 250 ± 25 g). All experiments were done under anesthesia with intraperitoneal injection of 50 mg/kg pentobarbital sodium after 24-h starvation. A small silastic catheter was placed into the right jugular vein for collection of peripheral blood samples. After anesthetization, physiological saline or 1 ng/ml/kg ghrelin dissolved in physiological saline was infused into the portal vein (IP) or right femoral vein (IV) with a pump at the rate of 1 ml/hr from 0 to 40 min. 20% glucose (10 mg/kg per min) was administered into the portal vein (IPGTT) or femoral vein (IVGTT) from 10 to 40 min. After 10 min, 150 µg/kg/ml atropine methyl bromide (antagonist of muscarinic choliner-

gic receptors) or 10 µg/kg/ml phentolamine mesilate (alpha-adrenergic blocker) was co-infused with glucose and ghrelin. Blood samples (at 0, 5, 10, 15, 20, 30, 40 and 60 min) were immediately transferred to polypropylene tube containing EDTA-2Na (1 mg/ml) and aprotinin (500 KIU/ml final concentration). Plasma glucose levels were measured by the glucose oxidase method, and plasma insulin levels were measured by a radioimmunoassay with the double antibody technique for insulin, using rat insulin as standard.
Results: The insulin and glucose levels before glucose infusion were not modified by ghrelin IV or IP alone. Following IVGTT, plasma insulin levels were not different between the control (20% glucose only infusion into the jugular vein) and ghrelin IV group. Area under the curve of insulin responses from 10 to 40 min (ΣIRI/30 min) in IVGTT are 5082 ± 1794 in control and 7195 ± 1500 pmol/L in ghrelin IV group (p=0.2164). In IPGTT, ΣIRI/30 min was diminished by ghrelin IP compared with the control (3042 ± 1098 vs. 6432 ± 930 pmol/L, p=0.0411), while that was not diminished by ghelin IV infusion compared with the control (ΣIRI/30 min; 6966 ± 1938 vs. 6432 ± 930 pmol/L, p=0.2776). The insulin response in the ghrelin and atropine IP infusion group in IPGTT was similar to that in the ghrelin alone IP infusion group in IPGTT. Conversely, insulin response in ghrelin and phentolamine IP infusion group was recovered to that in the control in IPGTT.
Conclusion: The inhibitory effect of ghrelin on insulin response in IPGTT was not by its direct vascular perfusion to the pancreas but by its passage through the portal vein. This study indicated that the sympathetic afferent nerve is the major pathway conveying ghrelin's signals for inhibitory effect on insulin secretion to the pancreas in IPGTT.

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Endogenous ghrelin inhibits glucose-induced insulin release in the rat perfused pancreas
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Background and Aims: Ghrelin is a peptide isolated from the human and rat stomach as an endogenous ligand for growth hormone secretagogue receptor (GHS-R). mRNAs for both ghrelin and GHS-R are present in the pancreatic islets. We previously reported that glucose-induced insulin release was suppressed by ghrelin in the isolated rat pancreatic islets. To clarify the physiological role of the endogenous islet ghrelin in insulin release, we employed the perfusion system of isolated rat pancreas that retains the intact microcirculation in islets, while counteracting the action of endogenous ghrelin using specific ghrelin antiserum and GHS-R antagonist.
Materials and Methods: Pancreas was isolated from male Wistar rats (11–12 weeks of age, 250–270 g) and perfused according to the method by Grodsky with modifications. The perfusate consisted of modified Krebs-Ringer bicarbonate buffer (pH 7.4) containing 10 mM HEPES, 0.5% BSA, and 4% dextran T-70. The perfusate was continuously oxygenated and kept at 37 °C. The flow rate was 2.5 ml/min throughout measurements. Fractions were collected at 1 min intervals and assayed for insulin using a rat insulin radioimmunoassay kit.
Results: Both the first and second phases of 8.3 mM glucose-induced insulin release were significantly increased by a GHS-R antagonist [D-Lys^3]-GHRP-6 (1 µM), while at 2.8 mM glucose it had no effect. Moreover, antiserum against ghrelin, but not normal serum, increased glucose-induced insulin release by 70%. Conversely, exogenously administered ghrelin (10 nM) diminished both phases of glucose-induced insulin release. In contrast, 10 µM acetylcholine-induced insulin release and 25 mM K^+-induced insulin release at 2.8 mM glucose were little affected by ghrelin.
Conclusion: These results indicate that endogenous ghrelin inhibits glucose-induced insulin release under conditions preserving microcirculation in the pancreatic islets, suggesting a physiological insulinostatic role of ghrelin. The counteracting ability of ghrelin is greater for glucose than for acetylcholine and high K^+ stimulation.

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Orexin – a differentially regulates pancreatic insulin and glucagon secretion in rodents
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Background and Aim: Orexin-A (OXA) is a recently discovered neuropeptide which regulates food intake and energy homeostasis. OXA interacts with two specific G-protein coupled receptors OX1R and OX2R, binding with much higher affinity to OX1R. The role of OXA on insulin and glucagon secretion is still controversial. Therefore, we investigated the effects of OXA on glucagon and insulin secretion using perfused rat pancreas. Furthermore we characterized the expression of orexin receptors and the signal-transduction cascades in pancreatic glucagon and insulin secreting cells.
Methods: To characterize the effects of OXA on cellular level we used InR1-G9 and INS-1 cells as permanent models of pancreatic A- and B-cells. OX1R expression on mRNA and protein levels was investigated by RT-PCR and immunocytochemistry. Adenylate cyclase activity and intracellular Ca^{2+} accumulation were determined by a cyclic AMP-ELISA kit or fura-2 measurements, respectively. Quantitative proglucagon and proinsulin mRNA levels were determined by real-time PCR (normalization against beta-actin). In situ pancreas perfusion studies were performed on anaesthetized adult male rats. Concentrations of secreted glucagon and insulin were determined by rat glucagon RIA or ELISA-assay kits, respectively.
Results: Expression of OX1R was detected on mRNA and protein level in InR1-G9 and INS-1 cells. Following OXA administration intracellular cAMP and Ca^{2+}concentrations increased in INS-1 cells and decreased in InR1-G9 cells. Consistent with the differential effects on both intracellular mediators of OX1R-signalling, OXA stimulated proinsulin mRNA-levels after 24 hours of incubation. In contrast, proglucagon gene expression was dramatically reduced, already after 8 hours of incubation with OXA. In agreement with these observations, OXA induced insulin secretion from INS-1 and from perfused rat pancreas, whereas glucagon secretion from InR1-G9 from perfused pancreas was reduced.
Conclusions: Our study provides the first in vitro evidence for the differential effect of OXA on pancreatic insulin and glucagon secretion. In addition to the description of OX1R-dependent signal transduction cascades this study shows potent effects of OXA on proglucagon and proinsulin gene expression. Since glucagon and insulin play a role in regulating food intake, the results of our study suggest that the orexigenic effects of OXA in rodents may be mediated by modulation of insulin and glucagon secretion.

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Orexin-A inhibits glucose stimulated insulin secretion in rat insulinoma cell line INS-1E
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Background and Aims: Orexins are neuropeptides involved in the regulation of feeding, sleep and autonomic functions. However, orexins are also detected outside the central nervous system in peripheral organs like the adrenals, neuroendocrine cells of the intestine and the endocrine pancreas. Yet, their effects on insulin secretion are still unclear.
Materials and Methods: mRNA from rat insulinoma β-cell line INS-1E was isolated and reverse transcribed to cDNA to study the expression of prepro-orexin and orexin-1 receptor by RT-PCR. To investigate the effects of orexin-A on glucose stimulated insulin secretion, cells were preincubated with orexin-A for 20 minutes before glucose was added for additional 30 minutes. Insulin was measured by ELISA. In order to study the signal transduction pathways involved, intracellular calcium ($[Ca^{2+}]_i$) was measured with a fura-2 based calcium imaging method. For calcium imaging, glass coverslips with adhered cells were loaded with the fluorescent calcium indicator fura-2 acetoxymethyl ester (4 µM, 30 min, 37°C) and excited by alternating wavelengths of 340 and 380 nm. Emission was measured through a 430 nm dichroic mirror and a 510 nm barrier filter. Ratios of fluorescence from 340 and 380 nm exposures were calculated.
Results: Prepro-orexin and orexin-1 receptor mRNA were expressed in INS-1E cells. Orexin-A significantly and dose-dependently inhibited glucose stimulated insulin secretion from INS-1E cells. At 5,5 mM glucose, 100 nM orexin-A reduced insulin secretion by approximately 30% whereas 500 nM orexin-A significantly inhibited insulin secretion (app. 48% inhibition, $p < 0,01$). At 16,7 mM glucose, insulin secretion was significantly inhibited by 100 nM orexin-A (app. 33% inhibition, $p < 0,05$) and 500 nM orexin-A (app. 44% inhibition, $p < 0,001$). In addition, the specific orexin-1 receptor antagonist SB-334867 (GlaxoSmithKline) reversed 100 nM and 500 nM orexin-A induced inhibition of insulin secretion at 16,7 mM glucose. Moreover, 100 nM orexin-A significantly reduced the elevation of $[Ca^{2+}]_i$ in response to 10 mM or 16,7 mM glucose in INS-1E cells ($p < 0,05$).
Conclusion: Our results suggest that orexin-A has an inhibitory effect on insulin secretion in INS-1E cells via a decrease in intracellular calcium.

Orexin-A might thus participate in the regulation of insulin secretion in a paracrine manner inside the islets.
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Central leptin modulates insulin secretion mainly through sympathetic nervous system
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Background and Aims: It is suggested that central leptin increases glucose utilization in muscle by increasing acitivity of sympathetic nervous system (SNS) in muscle and decreases insulin secretion. However, it is not elucidated that central leptin alter β-cell function and mass directly via SNS in pancreas or indirectly as results of altered insulin resistant states.
Materials and Methods: Male Sprague Dawley rats were randomly divided into two groups, sympathectomy in pancreas by phenol (SNSX) and sham operation (Sham). Each group was divided into four groups and it was intracerebroventricularly (ICV) or intravenously (IV) infused with leptin (1.33nmol/5 hrs or 5 μg/kg/min, respectively), or saline in chronically catheterized conscious rats. During leptin or saline infusion, glucose stimulated insulin secretion was measured by hyperglycemic clamp. And they were infused with ICV leptin (3 μg/day), ICV saline, IV leptin (10 μg/day) or IV saline by osmotic pump for 4 weeks. They were provided by 20 En% fat diets
Results: Body weight and fasting serum glucose levels were not different among groups in a long-term infusion study. Fasting insulin levels were decreased by ICV and IV leptin infusion. Acute ICV leptin administration in Sham rats suppressed the first and second phase insulin secretion at hyperglycemic clamp by about 48% compared to the ICV saline. However, acute ICV leptin in SNSX rats did not inhibit insulin secretion in both phases. In both SNSX and Sham rats, acute IV leptin decreased insulin secretion by about 23% with about 6 fold increase compared to saline administration. In SNSX, one month administration of ICV leptin improved hepatic and muscle insulin sensitivity, compared to that of ICV saline, while it suppressed the first and second phase insulin secretion in Sham rats compared to SNSX rats. However, the pancreatic β-cell mass was not changed by long-term administration of ICV leptin.
Conclusion: Leptin directly decreases serum insulin levels by a predominantly central mechanism, via the activation of pancreatic SNS, without modulating pancreatic β-cell mass. Leptin modulates not only insulin sensitivity in muscle but also insulin secretion by SNS.

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Glucokinase and Na^+-K^+-pump contribute to glucose-sensing in neuropeptide Y neurons in the rat hypothalamic arcuate nucleus
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Background and Aims: Glucose-sensitive (GS) neurons, the neurons that are activated by lowering glucose concentrations, are distributed in the brain. We have previously shown that a fraction of neuropeptide Y (NPY) neurons in the hypothalamic arcuate nucleus (ARC) are GS neurons. Since the GS NPY neurons are implicated in the regulation of feeding and glucose metabolism, their glucose-sensing mechanism is of (patho)physiological interest. The present study aimed to determine the involvement of glucokinase, energy production and Na^+-K^+-ATPase (pump) in the glucose-sensing process in the ARC NPY neurons.
Materials and Methods: Single neurons were isolated from the ARC of male Wistar rats aged 6–8 weeks. Cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_i$) and NAD(P)H in isolated single neurons were measured by fura-2 and auto-fluorescence imaging, respectively, followed by identification of NPY neurons by immunocytochemical staining with anti-NPY antiserum.
Results: Lowering the perfusate glucose concentration from 8.3 mM (high glucose; HG) to 2.8 mM (low glucose; LG) increased $[Ca^{2+}]_i$ in 20% of isolated neurons from the ARC, exhibiting the property of GS neurons. More than 90% of these GS neurons contained NPY. We examined whether the LG-induced increases in $[Ca^{2+}]_i$ in the GS NPY neurons were mediated by reductions of glucose availability, energy production and Na^+-K^+-pump activity and consequent depolarization-triggerd Ca^{2+} entry. A glucokinase inhibitor mannoheptulose (10 mM) and a mitochondrial uncoupler FCCP (30 nM) both increased $[Ca^{2+}]_i$ in HG conditions, mimicking the effect of LG. Administration of ourbain and deprivation of external K^+, the conditions that block Na^+-K^+-pump, also increased $[Ca^{2+}]_i$ at HG. Furthermore, LG reduced the NAD(P)H level. LG-induced increase in $[Ca^{2+}]_i$ was inhibited partially by nifedipine, a blocked of voltage-dependent Ca^{2+} channels.
Conclusion: The results provide evidence to support that the glucose-sensing in NPY neurons requires sequential operation of lowered glucose metabolism, lowered energy production, lowered Na^+-K^+-pump activity, consequent increase in voltage-dependent Ca^{2+} influx and resultant neuronal activation. It is suggested that glucokinase serves as an initial glucose sensor while Na^+-K^+-pump links the metabolic change to membrane potential and $[Ca^{2+}]_i$.

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Modulation of hippocampally-mediated cognition by insulin
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Background and Aims: Whether insulin plays a physiological role in brain function, particularly beyond involvement in control of feeding, remains controversial. The majority of studies to date have focussed on potential roles for insulin in the hypothalamus, in control of feeding (or energy regulation). However, the hippocampus is also replete with insulin receptors, and is known to respond to administration of glucose (which has marked cognitive effects) as well as to modulation of K-ATP channels which are known to be insulin-responsive. Moreover, hippocampal insulin receptor membrane concentrations have been suggested to respond to spatial memory tasks. Hence, we hypothesized that insulin might act to modulate hippocampal cognitive function, a possibility which has not been previously investigated.
Materials and Methods: We delivered insulin, KATP-modulating drugs, or PI3K inhibitors via microinjection into the left hippocampus, 10 min prior to a spatial working memory task. Injection volume was 0.5 uL for all treatments.
Results: Insulin showed an inverted-U dose response curve, with doses of 100 uU and 1 mU (in a volume of 0.25 uL) both producing a significant enhancement of task performance (all *p*s <.01). However, the KATP-opening drug diazoxide produced a dose-dependent deficit in performance following intrahippocampal injection ($p < .01$ for the highest dose used, 2.5 mM). Further, neither intrahippocampal IGF-1 nor insulin delivered to another brain region (the striatum) affected cognitive performance. In contrast, co-administering wortmannin with insulin fully reversed the cognitive benefit of insulin. In separate experiments, insulin was delivered via microdialysis, allowing measurement of local hippocampal glucose and lactate throughout insulin administration and task performance and hence evaluation of the impact of acute insulin delivery on local metabolism.
Conclusion: Our data show that insulin can acutely enhance hippocampally-mediated cognitive processes, but that this role does not appear to be via KATPs; rather, insulin appears to acutely modulate local hippocampal metabolism via a PI3K-dependent mechanism.
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PPARs and thiazolidinediones

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Novel anti-diabetic compound BLX-1117 with no affinity to PPAR γ, increases glucose uptake in 3T3-L1 adipocytes

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Background and Aims: In recent years, a number of PPAR γ or dual PPAR-α,γ agonist compounds were developed, but because of the safety issues, further developements were terminated in the clinical stages. We have introduced a novel Rational Drug Design Approach (RDDA) to produce several amino acid conjugated small molecules for the treatment of Type-2 diabetes and its complications. They were first screened in glucose uptake assay in adipocytes and subsequently they were checked for cytotoxicity and adipogenesis assay for their PPAR γ affinity. BLX-1117 is an amino acid conjugated small molecule (MW<500 Da) that has distinct mode of action as compared to any known anti-diabetic compound. Once we establish its pharmacokinetics parameter, this compound will be screened in insulin resistant diabetic db/db mice for its *in vivo* efficacy.
Materials and methods: Briefly the 3T3-L1 cells were differentiated by the addition of differentiation cocktail (72 µg/ml insulin, 0.5 mM IBMX, 400ng/ml Dexamethasone) for 4 days and later fed with media without differentation cocktail for 7–8 days. After differentiation the cells were incubated with the compound for 72 hours and carried out the glucose uptake for 10 min by the addition of KRP buffer supplememnted with 2.5 µCi/ml 14Cdeoxy glucose.
Adipogenesis was checked by the addition of the compound(0.1, 1 and 10 µM) and rosiglitazone(1 µM) for 9 days to the 3T3-L1 fibroblasts, replacing the compound and media for every 2 days. At the end the cells were stained with Oil Red O to check the extent of lipogenesis.
Toxicological studies was carried out by incubating the cells with the drug for 24 hours and viability tested by MTS (Formazan) assay.
Results: BLX-1117 is non-toxic even at higher concentrations(200 µM) in human liver cells and was effective in stimulating 14C-deoxyglucose uptake (200% of basal at 1 µM) compared to rosigltazone (195% of basal at 1 µM) in 3T3-L1 adipocytes upon treatment for 72 hours. Unlike the rosiglitazone, treatment with BLX-1117 for 9 days did not stimulate adipogenesis in 3T3-L1 fibroblasts as visualized microscopically after staining with Oil Red O. To quantify the amount of lipid accumulatin, Oil Red O was extracted by the addition of isopropanol and the absorbance was measured at 540 nm. Treatment with rosiglitazone significantly increased the lipogenesis(144% of basal at 1 µM), whereas no change observed in case of BLX-1117.
Conclusion: These results suggest that BLX-1117 is a novel anti-diabetic compound without any affinity to PPAR γ and not toxic to human liver (HepG2) cells even at higher concentrations. *In vivo* efficacy studies with BLX-1117 are in progress.

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A novel, balanced PPARalpha/gamma modulator (selective PPARgamma modulator plus potent PPARalpha agonist) improves metabolic abnormalities without inducing weight gain and edema in diabetic mice

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Background and Aims: PPARgamma full agonists, such as pioglitazone and rosiglitazone, have potential benefits in improving glycemic control in type 2 diabetic patients. However, administration of these agents causes weight gain, fluid retention and edema, which may lead to congestive heart failure. Expecting additional efficacy on associated dyslipidemia without causing such side effects, we have developed our original Compound A (CmpdA), which has unique chemical structure and activates both PPARalpha and gamma. In this study, we evaluated pharmacological efficacy of CmpdA in diabetic mice and characterized its action mechanisms.
Materials and Methods: Transactivation assays were conducted using monkey COS1 cells transiently transfected with a vector containing human PPARalpha or gamma ligand binding domain (LBD) as well as a vector containing a reporter gene. High-fat diet-induced obese mice or genetically diabetic *db/db* mice were orally treated with CmpdA and serum metabolic parameters were determined. Global gene expressions in the adipose tissue of *db/db* mice treated with CmpdA were analyzed using DNA chip. Glutathione-S-transferase (GST) pull down assay was performed using histidine-tagged human PPARgamma-LBD and TIF2-GST in the presence of CmpdA. Mouse 3T3-L1 adipocytes were treated with CmpdA and gene expressions were analyzed using real-time PCR.
Results: CmpdA activated human PPARalpha (EC_{50} = 0.62 microM) and human PPARgamma (EC_{50} = 0.81 microM) in the transactivation assays. Furthermore, CmpdA showed partial agonistic action to PPARgamma (intrinsic activity: 69% of pioglitazone). The pharmacological evaluation in diabetic models such as diet-induced obese mice and *db/db* mice showed that CmpdA as well as pioglitazone lowered blood glucose and plasma insulin. However, CmpdA, a dual activator of PPARalpha and gamma, decreased serum triglyceride more potently compared with pioglitazone. Interestingly, CmpdA at pharmacological dose did not induce either body weight gain or fluid retention, which was not the case with pioglitazone. In global gene expression analysis using adipose tissues of *db/db* mice treated with CmpdA or pioglitazone, patterns of expressions of PPARgamma-targeted genes were distinctly different between CpmdA- and pioglitazone-treated groups. In order to unveil molecular mechanisms on pharmacological actions of CmpdA, cofactor recruitments to PPARgamma were examined in the pull down assay. Interestingly, CmpdA did not affect the interaction of PPARgamma with TIF2, while pioglitazone potently enhanced this interaction. CmpdA may not either enhance recruitment of obesity-related cofactors, such as TIF2, or regulate downstream gene expressions. In 3T3-L1 adipocytes, CmpdA showed a distinct pattern of gene expression from that of pioglitazone, and did not enhance expressions of genes, such as aP2, being involved in deterioration of obesity.
Conclusion: CmpdA regulates the expression of a portion of PPARgamma-targeted genes selectively, possibly due to different manner of cofactor recruitment from PPARgamma full agonists. A balanced PPARalpha/gamma modulator, CmpdA, may offer preferable anti-diabetic and lipid-lowering effects with inducing less side effects.

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Effects of LBM642, a dual PPARα/γ agonist, on ectopic fat deposition in fatty Zucker rats

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Background and Aims: PPARγ activation leads to increased fatty acid (FA) uptake (i.e. hypolipemic action) in the adipose tissue and subsequently leads to the utilization of glucose instead of FAs as the energy source by the muscle and the liver (i.e. hypoglycemic action). In contrast, PPARα activation stimulates FA oxidation in skeletal muscles, adipose tissues and liver (i.e. anti-obesity action), but it has more limited effects on glucose uptake and glucose oxidation. Here, a comprehensive study was conducted to evaluate whether a dual PPARα/γ agonist (LBM642), improves glucose tolerance without inducing the common adverse effect of PPARγ on increased adiposity and body weight (BW) gain.
Materials and Methods: Four groups of fatty (*fa/fa*) Zucker rats (1. Vehicle, 2. PPARα fenofibrate 150 mg/kg, 3. PPARγ pioglitazone 30 mg/kg, 4. LBM642 5 mg/kg; n=10 for each group) were imaged under anesthesia both at baseline and after 4 weeks of treatment. All animals were fed with a 60% kcal fat diet (~90% from lard) for 6 weeks, with a 2-week lead-in and a 4-week treatment period. Whole-body insulin sensitivity index was determined pre- and post-treatment by the oral glucose tolerance test (OGTT). *In vivo* proton MR spectroscopy (^{1}H-MRS) and imaging (MRI) were applied under anesthesia pre- and post-treatment to determine hepatic and intramyocellular (IMCL) lipid accumulation, liver volume and whole-body adiposity. Treatment effects on lipid metabolism were further characterized by *ex vivo* NMR metabonomic analysis of liver extracts.
Results: Not only both fenofibrate and LBM642 prevented BW gain, independently of food intake, but also LBM642 was about as effective as pioglitazone at improving glucose tolerance over the treatment period. Both fenofibrate and LBM642 treatments led to a two-fold greater increase ($p<0.05$ vs. vehicle) in liver volume, which was likely due to a rodent-specific α-agonist effect on peroxisome proliferation. Supporting this, metabonomic analysis of liver tissues identified the elevation of a number of metabolites consistent with peroxisome proliferation in these two groups. The greater BW gain in the pioglitazone group ($p<0.05$ vs. vehicle) was attributed to increased adiposity predominantly in the visceral region. Conversely, a net loss of subcutaneous (SC) fat and a slight decrease in visceral fat contributed to a lower BW ($p<0.05$ vs. vehicle) in the fenofibrate and LBM642 groups. LBM642 appeared to be the most efficient of the three drugs at reducing total hepatic lipids ($p<0.05$, LBM642: -2.1 ± 0.8 vs. vehicle: $+11.1 \pm 2.6$, Feno: $+2.6 \pm 1.3$ and Pio: $+4.3 \pm 1.4$ mmol/liver), suggesting an α/γ synergistic effect on hepatic steatosis. In addition, *ex vivo* NMR showed that Pioglitazone treatment led to higher levels of monounsaturated fats, while LBM642 increased the relative amount of beneficial polyunsaturated fats. Finally, all three

treatments were equally effective at reducing IMCL contents to normal levels in the tibialis anterior muscle.
Conclusion: This study showed that LBM642 leads to improved glucose tolerance without concomitant weight gain. These results also helped to define LBM642 as a valid option to treat nonalcoholic fatty liver disease. Furthermore, *in vivo* measurement of lipid deposition in various tissues provided relevant readouts (i.e. IMCL, hepatic lipids, fat distribution) to be considered for drug profiling studies, including human proof-of-concept studies.

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Thiazolidinediones control the expression of phosphoprotein enriched in diabetes (ped/pea-15) both in vitro and in vivo
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Background and Aims: Overexpression of the ped/pea-15 gene is a common abnormality in type 2 diabetes. In cultured cells and in vivo, ped/pea-15 overexpression impairs insulin activation of protein kinase C zeta (PKCzeta) and of glucose disposal. Indeed, transgenic mice overexpressing ped/pea-15 show peripheral insulin resistance and reduced glucose tolerance. To address the significance of ped/pea-15 as a novel potential therapeutic target, we have investigated the effect of the PPARgamma agonists thiazolidinediones (TZDs) on ped/pea-15 expression
Materials and Methods: We have investigated this issue in cultured L6 skeletal muscle cells.
Results: In L6 skeletal muscle cells, the TZDs rosiglitazone and troglitazone decreased the endogenous ped/pea-15 protein expression leading to activation of PKCzeta and increased 2-DG uptake. TZDs also reduced ped/pea-15 mRNA levels by 40% ($p<0.001$). TZDs effect was not mimicked either by the biguanide metformin or the sulphonylurea glyburide. L6 cells stably transfected with a vector encoding ped/pea-15 full-lenght cDNA under the control of the cytomegalovirus promoter (L6 PED) showed no decrease of ped/pea-15 levels in presence of TZDs, suggesting a specific-TZD regulatory action on the ped/pea-15 promoter. Also PKCzeta and 2-DG uptake were not stimulated by TZDs in L6 PED cells. Consistent with these data in the isolated cells, muscle tissue from control mice showed a 90% decrease ($p<0.001$) of ped/pea-15 levels upon a 10 day treatment with rosiglitazone (26 mg/Kg/die). However, after TZDs administration, no differences were detected in ped/pea-15 expression in transgenic mice overexpressing ped/pea-15 under the control of human beta-actin promoter. Furthermore, incubation of the cells with the irreversible PPARgamma antagonist, GW9662, blocked the effect of rosiglitazone on ped/pea-15 through PPARgamma.
Conclusion: TZDs, but not biguanides or sulfonylureas, reduce ped/pea-15 expression. At least in part, TZDs effect on insulin sensitivity in glucose disposal may be mediated by reduction of ped/pea-15 levels.

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Rosiglitazone activates 5'-AMP-activated protein kinase and improves defective insulin activation of atypical protein kinase C (aPKC) in muscle of obese monkeys. Conserved insulin signaling in obese liver
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Background and Aims: Insulin signaling to insulin receptor substrate (IRS)-1-dependent phosphatidylinositol (PI) 3-kinase (IRS-1/PI3K), protein kinase B (PKB) and atypical protein kinase C (aPKC), and the direct activation of aPKC by the lipid product of PI3K, PI-3,4,5-(PO4)3 (PIP3), are defective in skeletal muscle (SM) of obese prediabetic monkeys (Diabetes 51:2936, 2002). However, it is unknown whether insulin signaling is similarly compromised in the liver of obese monkeys. In type 2 diabetic rats, thiazolidinediones (TZDs) improve insulin signaling to aPKC, but the mechanism is uncertain, and information on TZD action in obesity, prior to development of diabetes, is lacking.
Materials and Methods: SM (vastus lateralis) samples were obtained from 5 obese insulin-resistant monkeys before and during a euglycemic hyperinsulinemic clamp at baseline and after a dose escalation study with rosiglitazone (supplied by SmithKline Beecham) at 0.03, 0.1 and 0.3 mg/kg/day (4-weeks each dose). Liver samples were also obtained from obese insulin-resistant monkeys and young lean monkeys before and after the euglycemic hyperinsulinemic clamp.
Results: In SM, the TZD Rosiglitazone improved defects in insulin signaling to aPKC, but not to IRS-1/PI3K or PKB. In conjunction with improved SM aPKC activation by insulin administration in vivo, the responsiveness of aPKC to PIP3 in vitro was improved in obese monkeys following Rosiglitazone treatment. With respect to factors potentially relevant to improved responsiveness of SM aPKC to PIP3, Rosiglitazone increased plasma adiponectin levels more than 5-fold (mean±SE, 2.2 ± 0.36 vs. 11.9 ± 1.6 µg/ml, $p<0.002$), significantly improved VLDL-associated hypertriglyceridemia and hyper-fatty-acidemia, significantly decreased fasting plasma insulin, and activated 5'-AMP-activated protein kinase (357 ± 49 vs. 703 ± 27 cpm/immunoprecipitated, $p<0.001$) in SM of obese monkeys. In addition, SM CPT-1 mRNA expression was higher during the clamp following Rosiglitazone compared to baseline (baseline vs. Rosiglitazone, 1.3 ± 0.43 vs. 2.3 ± 0.53 mRNA/36B4, $p<0.005$). Of particular interest, unlike the insulin signaling defects seen in SM, the activation of IRS-1/PI3K, IRS-2/PI3K, PKB and aPKC by insulin was not significantly compromised in liver of obese monkeys.
Conclusion: Our findings provide initial evidence that, in insulin-resistant obese monkeys, insulin signaling is, as expected, impaired in SM, but surprisingly largely conserved in the liver. Thus, "hepatic insulin resistance" is not due to impaired activation of signaling pathways, and probably reflects modulation by non-signaling metabolic factors. Moreover, TZDs improve insulin signaling to aPKC in monkey SM by increasing aPKC responsiveness to PIP3, even in the absence of alterations in the activation of IRS-1-dependent PI3K and PKB. We hypothesize that increased responsiveness of aPKC to PIP3 is due to increases in plasma adiponectin and activation of SM AMPK along with an increase in SM CPT-1 mRNA expression, with subsequent improvement in fatty acid oxidation and the lipid environment in SM.

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Visceral adipose tissue in nonobese patients with new onset type 2 diabetes mellitus before and after combination therapy with rosiglitazone and fenofibrate
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Background and Aims: Obesity and type 2 diabetes mellitus are characterized by insulin resistance. However the metabolic characteristics of lean type 2 diabetic patients are not clearly defined. We determined the relationship between insulin resistance and visceral adipose tissue (VAT) in nonobese patients with new onset type 2 diabetes before and after one year treatment with the combination therapy of rosiglitazone and fenofibrate.
Materials and Methods: A number of 30 new onset type 2 diabetes patients and 20 healthy control subjects with similar features, between 45–72 years old, BMI <27 kg/m^2, C-peptide >0.6 nmol/L included in study. Only patient group received rosiglitazone 4 mg/day and fenofibrate 200 mg/day for a year. Fasting blood glucose, HbA1c, serum lipids, BMI, insulin, C-peptide, HOMA-IR and visceral and subcutaneous adipose tissue (by computed tomography) were measured at the beginning and after one year treatment.
Results: At the beginning, in patient group VAT was significantly higher than healthy control group ($33.17 \pm 10.23\%$ vs $16.53 \pm 7.85\%$, $p<0.001$). In patient group VAT was correlated with HOMA-IR ($r=0.62$, $p=0.003$). After one year treatment period, VAT significantly reduced in patient group compared to baseline ($33.17 \pm 10.23\%$ vs $22.89 \pm 6.11\%$, $p<0.001$), whereas in healthy control subjects no significant difference was observed ($16.53 \pm 7.85\%$ vs $17.61 \pm 6.19\%$, $p>0.05$). HOMA-IR reduced significantly after treatment in patient group (3.82 ± 2.22 vs 1.93 ± 0.98, $p<0.001$), while the change in HOMA-IR showed no significance in healthy control subjects.
Conclusion: The amount of VAT is significantly higher in nonobese new onset patients with type 2 diabetes than the healthy control group. In these patients VAT measured by CT is an important indicator of insulin resistance. Treatment with dual PPAR gamma/alpha stimulation with combination of rosiglitazone and fenofibrate effectively decreases VAT and improves insulin resistance.

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Adiponectin, but not a reduction in oxidative stress, contributes to the beneficial effect of rosiglitazone on vascular function in type 2 diabetic patients

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Background and Aims: Improvement in both endothelium-dependent and endothelium-independent vasodilation has been observed following treatment with rosiglitzone (ROS), an insulin sensitizing agent, in type 2 diabetic patients. As hypoadiponectinaemia and increased oxidative stress have been implicated in the pathogenesis of vascular dysfunction in type 2 diabetes, we investigated whether the beneficial effect of ROS was mediated through changes in circulating adiponectin and/or a reduction in oxidative stress.
Materials and Methods: 51 type 2 diabetic patients with suboptimal glycaemic control on sulphonylurea (SU) and metformin were randomized to add-on treatment with SU or ROS. Flow-mediated (endothelium-dependent) and glyceryl trinitrate-induced (endothelium-independent) vasodilation (FMD and NMD respectively) of the brachial artery were assessed by high-resolution ultrasound at baseline and 8 weeks after randomization. Adiponectin and MDA were measured using an in-house ELISA assay and the OXI-TEK TBARS assay kit respectively.
Results: After 8 weeks of add-on treatment with SU (n=25) or ROS (n=26), there was no significant difference in HbA1c (p=0.646) between the two groups, although mean HbA1c had decreased in both groups (p<0.001). HOMA-IR, however, was significantly reduced only in the ROS group (p<0.001). Both FMD (5.96 ± 3.07% versus 4.27 ± 2.00% at baseline; mean±SD; p=0.014) and NMD (16.4 ± 7.1% versus 14.2 ± 6.2% at baseline; p<0.001) improved significantly in the ROS group. In the SU group, a small improvement in NMD was observed (15.5 ± 5.8% versus 15.1 ± 4.9% at baseline; p<0.001), but there was no significant change in FMD. Serum adiponectin showed a marked rise in the ROS group (13.2 ± 9.8 versus 6.2 ± 3.2 μg/ml at baseline; p<0.001) and a small rise in the SU Group (7.3 ± 2.7 versus 6.5 ± 2.6 μg/ml at baseline; p<0.01). BMI showed no significant change on ROS but a small reduction was seen in the SU group (p<0.05). A significant fall in MDA level was seen only after ROS treatment (7.6 ± 1.7 versus 9.3 ± 1.5 nmol/ml at baseline; p<0.001). The % changes in FMD did not correlate with changes in adiponectin, MDA, HbA1c, HOMA-IR or BMI, but a significant correlation was found between the % changes in NMD and adiponection (r=0.344; p=0.013; n=51). Of all the parameters measured, % changes in MDA only correlated significantly with those in HOMA-IR (r=0.553; p<0.001).
Conclusion: Treatment with ROS was associated with a two-fold increase in serum adiponectin which probably contributed to the improvement in endothelium-independent vasodilation, but not the effect of ROS on endothelium-dependent vasodilation, in these patients with type 2 diabetes. The reduction in oxidative stress associated with ROS treatment was strongly related to its effect on insulin resistance but did not contribute significantly to its beneficial actions on vascular function.
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Comparison of the effects of rosiglitazone and metformin on inflammatory markers and adipokines: the role of adiponectin and interleukin-18 in the pharmacological effects of rosiglitazone

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Background and Aims: We examined the effects of rosiglitazone and metformin on the concentrations of inflammatory markers and adipokines in type 2 diabetic patients. In addition, we analyzed associations among the changes of inflammatory markers, adipokines, insulin sensitivity, and beta-cell function in the patients treated with rosiglitazone and sulfonylurea.
Materials and Methods: A total of 120 type 2 diabetic patients were randomized and treated with glymepiride and rosiglitazone or glymepiride and metformin for 12 weeks. Plasma concentrations of inflammatory markers and adipokines were measured at baseline and at week 12.
Results: The fasting insulin level, the QUICKI, and the HOMA-beta were improved in the rosiglitazone-treated group, but the QUICKI was only improved in the metformin-treated group. The adiponectin concentration was significantly greater after 12 weeks in the rosiglitazone-treated group. The rosiglitazone-treated group had significant decreases in the concentrations of resistin, C-reactive protein, tumor necrosis factor-alpha, interleukin-6 (IL-6), and IL-18. Metformin-treated patients did not show any such changes. The changes in the adiponectin concentration were significantly correlated with the changes in the QUICKI. The changes in IL-18 concentration were significantly correlated with the HOMA-beta.
Conclusion: We found that rosiglitazone but not metformin Improved the plasma concentrations of inflammatory markers and adipokines in type 2 diabetics. Our results suggest that rosiglitazone may increase beta-cell function, decrease insulin resistance, and decrease the risk of cardiovascular disease via effects on inflammatory markers and adipokines.
This study was supported by a grant of the Brain Korea 21 project, Yonsei university

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Randomized trial to compare the effects of rosiglitazone and metformin on clinical, metabolic and vascular parameters in patients with HIV-lipodystrophy

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Objective: The use of highly active antiretroviral therapy (HAART) in HIV has been associated with lipodystrophy and metabolic risk factors that increase the risk for cardiovascular disease. We aimed to compare the effects of the peroxisome proliferator-activated receptor-γ agonist rosiglitazone and metformin on body fat distribution, insulin sensitivity and endothelial function in patients with HIV-lipodystrophy.
Methods and results: Thirty-nine patients were ranodmly assigned to receive rosiglitazone (8 mg/d) and metformin (2 g/d) for 26 weeks. Rosiglitazone selectively increased subcutaneous abdominal fat (+16%), while metformin decreased subcutaneous (-11%) and visceral (-14%) abdominal fat. The area under the curves (AUCs) for glucose and insulin after the OGTT decreased with both rosiglitazone (-17% and -34%, respectively, p<0.01) and metformin (-8% and -30%, respectively, p<0.05). Adiponectin markedly increased with rosiglitazone (+116%, p<0.01), but not with metformin (-8%). Rosiglitazone significantly increased fasting plasma triglycerides, while metformin showed a modest improvement of lipid profile. FMD was significantly increased with metformin (from 3.9 ± 0.7 to 5.5 ± 0.6%, p<0.05), but not with rosiglitazone (from 4.5 ± 0.5 to 5.5 ± 0.8%). C-reactive protein did not change with either rosiglitazone or metformin.
Conclusions: The differential effects of rosiglitazone and metformin on cosmetic, metabolic and vascular parameters reinforces the importance of individualized care in HIV-infected patients. Although rosiglitazone may partly correct lipoatrophy, metformin has benefits on fasting lipid profile and vascular function, and may therefore be the drug of choice for reduction of cardiovascular risk in this population.
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Utility of the diabetes risk score to identify persons with impaired fasting glucose

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Background and Aims: One major problem in diabetes prevention is to identify persons at risk who will profit from preventive steps. We assessed the utility of the diabetes risk score by Lindström and Tuomilehto to identify persons with impaired fasting glucose and undetected diabetes.
Materials and Methods: We invited employees of a big industrial company and employees of the community to complete a questionnaire designed to assess their diabetes risk. According to the recommendation, persons with a risk score > 9 were invited to determine their fasting blood glucose.
Results: From a total of approx. 10.000 persons, who were invited to participate in the screening performed by the risk questionnaire, 845 persons completed this questionnaire (approx. 8.4%). From these persons, 434 had a risk score >9. Fasting blood glucose could be obtained from 391 (90%). From these, 53 persons (13.5%) fulfilled criteria for impaired fasting glucose and 26 persons fulfilled criteria for diabetes (6.6%). Sensitivity, specificity, positive and negative predictive values of the diabetes risk score to predict current impaired fasting blood glucose or diabetes are shown in table 1. The ROC area under the curve was .646.
Conclusion: The rate of participants completing the questionnaire was rather low. If an elevated risk score was identified, attrition rate to measuring fasting blood glucose was high. The diabetes risk score was designed to predict the risk of diabetes in the future. The screening abilities of this risk score to identify persons with present impaired fasting glucose or diabetes was rather modest in our sample, as indicated by the ROC area under the curve. The suggested cut-off for screening (Score > 9) will lead to a great number of negative test results. At the cost of sensitivity, a higher cut-off score of >=14 or => 16 could be more appropriate to avoid unnecessary blood glucose testing

Screening characteristics of different cut-off scores of the diabetes risk score

	=>10	=>12	=>14	=>16	=>18	=>20	=>22
Sensitivity	100	79.4	58.2	48.2	32.9	16.4	6.3
Specificity	0	31.7	59.6	82.7	94.2	97.8	98.7
ppv	20.5	22.8	26.7	41.3	59.1	65	55.5
npv	100	86.1	84.9	86.3	84.7	82.2	80.6

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Impaired fasting glucose (IFG) as a risk factor for diabetes – five year follow-up

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Background and Aims: In November 2003 American Diabetes Association changed the lower threshold for impaired fasting glucose (IFG) from 110 to 100 mg/dl. This prospective study aimed at assessing the prevalence of diabetes in redefined (100–125 mg/dl) IFG subjects.
Materials and Methods: The study cohort consists of 297 individuals (mean age 63 ± 11 years): 114 subjects with IFG, 138 with impaired glucose tolerance (IGT), 45 with concomitant IFG and IGT (IFG/IGT). The controls are age- and gender-matched 150 individuals with normal glucose tolerance. In all subjects 75 g oral glucose tolerance test (OGTT) is performed at one-year intervals. The subjects were informed about OGTT results and the risk associated with IFG or IGT status. Moreover, at study entry standard lifestyle modification advice (changes in diet and increase in exercise) was provided. No other intervention was conducted.
Results: One hundred and eight individuals completed five-year follow-up period: 41 with IFG, 16 with IFG/IGT, 15 with IGT and 36 controls. In 18 (44%) subjects from IFG group glucose metabolism returned to normal, while in 8 (20%) IFG remained. Diabetes was found in 15 (36%) subjects from this group, no person was found to have IGT. Subjects with diabetes had significantly greater baseline body weight than the subjects in whom normal glucose metabolism was restored: 89.3 ± 11.7 vs 80.4 ± 11.9 kg; BMI 31.8 ± 2.9 vs 29.2 ± 2.4 kg/m^2 ($p<0.05$). In IFG/IGT group 8 (50%) subjects had normal glucose tolerance, 3 (19%) subjects had IFG, and 5 (31%) developed diabetes. In 9 (60%) persons from IGT group glucose values returned to normal, and 6 (40%) developed IFG. 14 (39%) controls remained healthy, IFG was found in 20 (55%) and diabetes in 2 (6%) controls.
Conclusion: Given these preliminary results, it may be assumed that IFG may resolve in up to 50% of subjects within 5-year period, without conducting specific intervention. Moreover, it seems that IFG is a significant risk factor for diabetes rather than for IGT development, particularly in obese subjects.
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Elevated fasting glucose is associated with higher medical care costs

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Background and Aims: In 2003, the American Diabetes Association reduced its cut-point for defining impaired fasting glucose (IFG) from 6.1 to 5.6 mmol/l. Our objective was to estimate medical costs associated with elevated fasting plasma glucose (FPG), and to determine whether costs differed for patients who met the 2003 versus the 1997 ADA cut-point for IFG.
Materials and Methods: We identified 28,335 patients with two or more FPG test results of at least 5.6 mmol/l between 1 January 1994 and 31 December 2003. Subjects with evidence of diabetes before the second test were excluded. We categorized patients into two stages of abnormal glucose: 5.6–6.0 mmol/l (stage 1) and 6.1–6.9 mmol/l (stage 2), and matched each subject to a patient with a normal FPG test (<5.6 mmol/l) on age, sex, and year of FPG test. All subjects were followed until an FPG test qualified them for a higher stage, an anti-hyperglycemic drug dispense, health plan termination, or 31 December 2003. We further divided the 26,309 control subjects who had not died by the end of 2003 into 1) those who had no FPG test ≥ 5.6 mmol/l (n=23,621); 2) had progressed to stage 1 FPG (n=1,741); 3) had progressed to stage 2 (n=462); or 4)had progressed to diabetes (n=485). Cost coefficients were applied uniformly in all years, inflated to 2003 dollars. To minimize the effects of censoring, we annualized costs by dividing by months of observation, and then multiplying by 12. Using diagnoses in the electronic medical record, we identified comorbidities present at the time of the index FPG test. We adjusted costs for age, sex, and history of MI, stroke, other cardiovascular disease (CVD), congestive heart failure (CHF), and depression. Adjusted costs were then weighted by months of observation.
Results: Subjects averaged 59 years of age, and 54% were women. CVD or CHF was present in 20.8% of subjects with normal FPG, compared to 26.7% in stages 1 and 30.4% in stage 2 ($p<.001$, all comparisons). Adjusted annual costs were $4,357 among patients with normal FPG, $4,580 among stage 1 patients, and $4,960 among stage 2 patients ($p<.001$, all comparisons). Among normal FPG subjects, however, those who did not progress incurred significantly lower costs ($3,755) than subjects who ultimately progressed to diabetes ($6,796, $p<0.001$) or progressed to stage 2 FPG ($4,488, $p=0.025$). After removing normal FPG patients who subsequently developed abnormal glycemia, costs in the normal FPG stage were $3,799.
Conclusion: Our results demonstrate that elevated fasting glucose is associated with higher medical care costs. The 2003 ADA cut-point of 5.6 mmol/l identifies a group of patients with greater costs and comorbidity than normo-glycemic patients, but with lower costs and less comorbidity than patients above the 1997 cut-point (6.1 mmol/l). For patients identified with IFG by the new ADA definition, elevated FPG is not in itself associated with higher medical costs, primarily because these patients are more likely to have CVD. However, CVD-adjusted costs remained significantly higher for stage 2 IFG, suggesting that, at higher levels, abnormal glucose metabolism does add independently to costs. Among subjects with normal FPG, current costs may predict future abnormal glucose metabolism.
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Long-term prospective study on new-onset diabetes mellitus in Korean renal allograft recipients

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Background and Aims: The incidence of new-onset diabetes mellitus (DM) after renal transplantation has been either underestimated due to lack of a standard definition or overestimated due to a short observation period. This prospective longitudinal study was performed to investigate the incidence and risk factors of posttransplantation DM (PTDM) in renal allograft recipients in Korea over a period of 7 years according to the criteria of American Diabetes Association.
Materials and Methods: A total of 97 non-diabetic renal allograft recipients taking corticosteroids and cyclosporine underwent 75-g oral glucose tolerance test (OGTT) at 0, 1, and 7 years after renal transplantation. Fasting, 1-, and 2-hour plasma insulin and glucose levels were measured and insulin secretion was estimated using the area under the curve (AUC)-insulin and the AUC-glucose. The OGTT-derived insulin sensitivity index was used as a surrogate estimate of insulin sensitivity.
Results: The incidence of PTDM was 41.2% after 1 year and 35.1% after 7 years, respectively. There was a significant decrease of insulin secretion in PTDM group compared with normal glucose tolerance group ($p = 0.04$ after 1 year and $p < 0.001$ after 7 years, respectively), whereas no significant difference in insulin sensitivity was observed (p = NS after 1 year and p = NS after 7 years, respectively). Old age ($p < 0.001$) and high immunosuppressant doses ($p < 0.05$) were independent risk factors of PTDM, while family history of DM, body mass index, and dyslipidemia were not associated with the development of PTDM.
Conclusion: These results show that impaired insulin secretion may play a crucial role in the development of PTDM. The incidence of PTDM after 7 years was less than that after 1 year after renal transplantation. This seems to be associated with tapering of immunosuppressant over time.

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Obesity is a major determinant of the association of C-reactive protein levels with the number of metabolic syndrome components in recently diagnosed, drug-naive type 2 diabetes: the ADOPT study cohort

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Background and Aims: C-reactive protein (CRP), a marker of systemic inflammation, is associated with long-term cardiovascular morbidity in patients with type 2 diabetes (T2DM) and in non-diabetic patients with the Metabolic Syndrome (MS). Elevated CRP has been found in non-diabetic obese subjects. The purpose of this study was to determine the contribution of body adiposity and glucose control to CRP levels in recently diagnosed (≤3 years), drug-naive, T2DM patients (fasting plasma glucose ≤10 mmol/l).
Materials and Methods: We examined a random representative subgroup (n=903) of the US cohort in ADOPT (A Diabetes Outcome Progression Trial). The relationship between baseline variables, National Cholesterol Education Program (NCEP) Adult Treatment Panel III MS phenotype and high-sensitivity CRP (hsCRP) levels was explored.
Results: Geometric mean hsCRP significantly increased with increasing numbers of MS components based on a test for linear trend ($P<0.0001$; Table). Similarly, BMI ($P<0.0001$) and HbA_{1c} ($P=0.0004$) increased with increasing numbers of MS components. Adjustment of CRP levels for body adiposity abolished the association between CRP and the number of MS components ($P=0.237$; Table), whereas adjustment for HbA_{1c} maintained the association ($P<0.0001$).

	# NCEP MS Components				
(N)	1 (30)	2 (94)	3 (219)	4 (332)	5 (228)
hsCRP*, mg/l	1.7 (1.1, 2.5)	2.4 (1.9, 3.0)	3.6 (3.1, 4.2)	4.0 (3.6, 4.5)	4.7 (4.1, 5.4)
BMI**, kg/m^2	25.5 (23.8, 27.1)	29.3 (28.3, 30.3)	33.1 (32.5, 33.8)	34.3 (33.8, 34.9)	36.1 (35.5, 36.7)
BMI-Adjusted CRP*, mg/l	3.2 (2.3, 4.7)	3.4 (2.8, 4.2)	3.8 (3.3, 4.3)	3.8 (3.4, 4.2)	3.9 (3.5, 4.5)

*Geometric Mean, **Mean, (95% CI)

Conclusion: We conclude that CRP, a marker of systemic inflammation, is strongly related to the number of MS components; however, in recently diagnosed, drug-naive T2DM patients this relationship is determined by body adiposity and not by glucose control.

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Ethnic differences in the metabolic syndrome among immigrants in Oslo, Norway

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Background and Aims: The emerging risk of the metabolic syndrome in industrialized countries can be justified by the obeseogenic environment and lifestyle typified by physical inactivity, over nutrition and stress. Our aim is to compare ethnic differences in the prevalence of the metabolic syndrome, in immigrants from developing countries exposed to such an environment.
Materials and Methods: The cross sectional, population based, Oslo Immigrant Health Study was conducted in 2002. Immigrants residing in Oslo aged 31–60 years from Turkey, Iran, Pakistan, Sri Lanka and Vietnam and were invited to the screening. The 2987 participants included here completed a postal health questionnaire and attended a clinical examination that included blood pressure, anthropometric measurements and a blood test. We used modified ATP-111 criteria (table 1), and established cut offs in the non fasting triglycerides and glucose based on the fasting samples.
Results: After adjusting for age, ethnic and gender differences were observed in the prevalence of the metabolic syndrome and its individual components ($p<0.001$). Pakistani women had the greatest proportions of those with abdominal obesity (65%) and Diabetes (20.3%) whereas Pakistani men those with hypertension (38.5%).The greatest proportions of high triglycerides were observed among Turkish men (37%) and low HDL among Sri Lankan women (66.4%).The greatest proportion of those with the metabolic syndrome were Pakistani men and women (35.3% and 34.9%) and those with the least proportion were men and women from Vietnam (10.4% and 5.8%). These ethnic differences persisted after adjusting for socio-demographic and lifestyle factors.
Conclusion: Immigrants from developing countries in Norway are no exception to the emerging risk of the metabolic syndrome as is evident among the five ethnic minorities in our study, particularly those from the Indian Subcontinent. Ethnic differences could only be partly explained by socio-demographic and lifestyle variables and need to be further studied.

Age Adjusted Ethnic Differences in the Metabolic Syndrome and its Components

		N	Waist Cir	High TG	Low HDL	Diabetes	Hyper-tension	Metabolic Syn
Men	Turkey	228	61	37	42	9	31	30
	Sri Lanka	603	45	35	46	14	35	26
	Iran	358	50	28	38	4	25	22
	Pakistan	244	60	34	46	15	39	35
	Vietnam	244	14	32	18	8	24	10
Women	Turkey	196	58	24	44	10	19	22
	Sri Lanka	398	47	27	66	14	22	25
	Iran	238	29	20	41	3	14	11
	Pakistan	196	65	30	60	20	22	35
	Vietnam	291	5	16	34	5	17	6
Cut Offs			>90 cmM >85 cmW	>2.7 mmolM >2.2 mmolW	<1.0 mmolM <1.3 mmolW	*self reported &Glucose >7.8M >7.6W*	*>=135/ 85mmhg & medi-cation*	

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Comparison of biological variation of insulin resistance markers

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Background and Aims: Insulin resistance is a marker of incipient type 2 diabetes and is used routinely to identify individuals who are at increased risk of developing this disorder. It is important that biochemical markers used in clinical practice are relatively stable, reproducible and easily measured. We carried out a study to compare the biological variation of several established surrogate markers of insulin resistance in subjects with the metabolic syndrome and in healthy controls.
Material and Methods: Fasting blood samples were collected at baseline and 10, 20, 30 days later from 10 subjects with the metabolic syndrome (5 males, 5 females, mean age 39.3 ± 11.6 yr) and 10 age- and sex-matched healthy controls (mean age 38.0 ± 11.0 yr). The samples were used to determine plasma glucose, insulin, adiponectin and sex hormone binding globulin (SHBG) concentrations. The homeostasis assessment model [HOMA] % sensitivity index was then calculated using the HOMA/CIGMA computer model. A nested ANOVA was used to compare the data in the two groups and also to determine the percentage biological variation of all the indices over the 30 day study period. The results of these analyses are summarised below.

Results:

	Metabolic Syndrome Mean(SD)	%Variation	Controls Mean(SD)	%Variation
Glucose (mmol/l)	5.4 (0.4)*	4.9%	5.1 (0.4)	4.3%
Insulin (pmol/l)	145 (116)*	24.6%	35 (18)	27.2%
HOMA (%S)	46 (24)*	27.0%	174 (122)	44.7%
Adiponectin (µg/ml)	3.7 (1.3)*	12.2%	6.3 (3.4)	18.8%
SHBG (nmol/l)	27.2 (13.3)*	10.1%	50.9 (32.0)	18.1%

* $p < 0.01$ for difference between metabolic syndrome and control groups.

Conclusion: The nested ANOVA showed there were significant differences in all the indices between the two groups. This analysis also demonstrated relatively large variations over time in plasma insulin, adiponectin and SHBG levels and HOMA % sensitivity index. The biological variation in these indices was lower in the metabolic syndrome group than in the control group. In both groups, the percentage variation over time in plasma adiponectin and SHBG levels was considerably lower than that measured for insulin and HOMA (%S). These results demonstrate that plasma adiponectin and SHBG have less biological variation than other markers of insulin resistance and therefore may be more reliable for serial assessment of insulin function in normoglycaemic individuals.
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Abnormalities of adiponectin and leptin in white European and South Asian subjects screened for impaired glucose tolerance and type 2 diabetes

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Background and Aims: Accumulating evidence suggests that inflammation is the link between obesity, type 2 diabetes and cardiovascular disease. Inflammatory markers that have been implicated are CRP, TNFα and IL-6. In addition, adipose tissue produces a number of adipocytokines such as adiponectin, leptin and resistin, which have been correlated with risk of developing abnormal glucose tolerance and cardiovascular disease. The South Asian (SA) population is known to have 2–4 fold increase in CVD compared with White Europeans (WE) although the exact mechanisms for this remain unclear. We studied the levels of these circulating risk markers in a cohort of WE and SA subjects diagnosed with IGT or T2DM through screening and compared them with age and sex matched controls. We sought to determine the level of risk as determined by concentration of these markers in each ethnic group across the glucose intolerance spectrum.
Materials and Methods: 217 subjects (110 WE, 107 SA) were recruited following OGTT screening for diabetes in Leicestershire, UK, through the STAR study (LREC approved). 61 subjects had diabetes of which 34 were WE (53% male), age 62 ± 10y (mean±SD), BMI 31.7 ± 4.8 kg/m^2, and 27 were SA (52% male), age 53 ± 11y, BMI 29.2 ± 5.5 kg/m^2. 92 subjects had IGT with 46 WE (46% male), age 60 ± 10y, BMI 29.7 ± 5.8 kg/m^2, and 46 SA (46% males), age 54 ± 10.6y, BMI 27.6 ± 4.3 kg/m^2. 64 were age and sex matched controls with 30 WE (53%), age 57 ± 11y, BMI 22.7 ± 1.5 kg/m^2, and 34 SA (47%), age 53 ± 11y, BMI 24.3 ± 2.9 kg/m^2. The samples were analysed using ELISA hsCRP assay and bioplex assays for determining adiponectin, leptin, resistin and TNFα.
Results: In both ethnic groups several inflammatory markers were altered in the IGT and diabetic states compared with controls. Leptin levels in both control groups were significantly different from each other (leptin: (Mean±SEM) WE: 4.01 ± 1.14 ng/mL vs SA: 28.41 ± 7.99 ng/mL, $p<0.001$). Leptin was also observed to increase progressively with IGT and T2DM in both SA and WE ($p>0.05$ and $p<0.05$ respectively), with a similar pattern noted with TNFα. Adiponectin significantly decreased in both ethnic groups with IGT and T2DM ($p<0.05$ for both ethnic groups). There was no significant increase in resistin, hsCRP, and TNFα levels with glucose intolerance in either ethnic group.
Conclusion: Our study suggests that altered levels of adipocytokines are seen in SA prior to development of glucose intolerant states, whereas in WE, these markers change significantly only on developing IGT and T2DM. We hypothesise that adipocytokine levels such as leptin and adiponectin may represent important key metabolic markers to determine early pathogenic risk for T2DM related complications such as CVD in different ethnic groups.

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Prediction and prevention of type 2 diabetes II

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Challenging established paradigms in the early development of insulin resistance

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Background and Aims: Cost-effective strategies for the prevention of diabetes depend on an understanding of causative factors. The current epidemic of childhood obesity and the insulin resistance associated with it is a recent phenomenon and, by implication, a product of environmental rather than genetic change. Accordingly, it is important that theories to explain it be drawn from data on contemporary children. EarlyBird is a non-intervention, prospective cohort study documenting lifestyle as well as physical and metabolic development of 300 healthy children from 5 years. It aims, specifically, to determine environmental as well as genetic factors that will lead some, but not others, to develop insulin resistance. Its findings question some established paradigms regarding its earliest development.
Materials and Methods: Observation of 300 healthy children and their parents. Main outcome measures include BMI, sum of 5 skinfolds, % body fat and fat distribution (DEXA), resting metabolic rate, dietary intake and physical activity (PA) by MTI accelerometer. Uniquely, serial fasting blood samples allow us to monitor both the behaviour of insulin resistance (IR) in young children and the progressive impact of IR on metabolic health (including lipids, glucose and blood pressure). We have archived all our sera and have a wealth of corresponding physical, social and metabolic data.
Results: A number of novel and unexpected findings have emerged from the school entry data and are summarized below:
1. Low birth weight is relatively rare in the westernized world and can no longer be held responsible for the increasing prevalence of diabetes in childhood. In contemporary UK 5-yr-olds, where birth weights are in fact rising rather than falling, IR is no longer inversely associated with birthweight ($r=0.02$), but correlates positively with current weight ($r=0.44$, $p<0.001$). Excess weight gain from the earliest years should be the principal target in prevention.
2. After correction for waist circumference, mothers are 25% more insulin resistant than fathers ($p<0.001$). Similarly girls are intrinsically 35% more insulin resistant than boys ($p<0.001$), even after controlling for higher adiposity and lower PA. The difference impacts significantly, and adversely, on metabolic status (triglycerides, HDL cholesterol and SHBG, all $p<0.01$), even at 5y, and may help explain the greater prevalence of type 2 diabetes among female adolescents.
3. A child's own PA level is strikingly consistent weekday-weekend $r=0.52$, year-on-year $r=0.50$, $p<0.001$.There is a four-fold variation in total PA between children, but timetabled PA at school (9 hr v 1.8 hr) explains <1% of the variance. The evidence suggests that a child's PA level may be under biological control and not amenable to environmental change.
4. Mean adiposity (% fat) rises progressively and significantly before puberty, yet IR and other markers for metabolic risk unexpectedly improve.
5. Overweight is increasingly seen as the norm - 60% of parents of overweight/obese children are both unaware of and unconcerned about their child's excess weight.
Conclusions: Cohort studies are the most powerful in epidemiology. Understanding the pathogenesis of insulin resistance from its earliest development is crucial to the prevention of diabetes. As a prospective, single cohort study, EarlyBird will continue to add to that understanding.
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Prognostic value of the metabolic syndrome for conversion of IGT to type 2 diabetes: epidemiological results of the STOP-NIDDM trial

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Background and Aims: Elevated fasting plasma glucose (IFG) and impaired glucose tolerance (IGT) are accepted risk categories for type 2 diabetes. IFG is a single trait of the metabolic syndrome (MS) according to NCEPIII criteria. So far only scarce information from prospective trials exist on the relative risk associated with single traits and the MS in people with IGT. STOP-NIDDM is a multinational prospective study in subjects with IGT. This report analyses the following questions:
(1) What is the hazard ratio (HR) for single traits in this high risk population?
(2) What adds the MS per NCEPIII definition to the risk of conversion to diabetes?
Materials and Methods: Subjects were recruited from a high risk population for diabetes aged 40–70 years with BMI greater or equal than 25 to 39. They were included if they had IGT by 75 g oGTT and fasting plasma glucose (FPG) greater or equal than 5.6 to smaller than 7.8 mmol/L. This analysis considers the patients of the placebo group (n=612) who had FPG less than 7.0 mmol/L at baseline. Median follow-up time in this subpopulation was 3.0 years. The single traits and prevalence of MS were determined according to NCEPIII criteria. The HR was calculated by Cox regression analysis stratified by country.
Results: The prevalence of traits of the MS was as follows: IFG 50%, large waist circumference 69%, low HDL cholesterol (HDL) 51%, hypertriglyceridemia 52%, elevated blood pressure 58%. The HR were as follows: IFG: 1.49, $p=0.003$, hypertriglyceridemia: 1.61, $p<0.001$, low HDL: 1.26, $p=0.105$, large waist circumference: 1.12, $p=0.441$ and elevated blood pressure: 1.06, $p=0.671$. The HR for the MS was 1.53, $p=0.002$. Among the triple combinations of the MS the highest HR was found for IFG + hypertriglyceridemia + large waist: 1.75, $p<0.001$, the lowest for hypertriglyceridemia + high BP + low HDL: HR=1.19, $p=0.271$.
Conclusion: Our results show striking differences for single traits as hazard for conversion to diabetes with IFG and hypertriglyceridemia as strongest predictors. Furthermore, the hazard due to the MS strongly depends on the combinations leading to diagnosis. The combination IFG + hypertriglyceridemia + large waist bears the highest risk.
Support: Research grant of Bayer AG

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Importance of white blood cell count and alanine aminotransferase as predictors of type 2 diabetes in subjects with impaired glucose tolerance: the STOP-NIDDM trial

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Background and Aims: Identifying individuals with pre-diabetes who have a high probability to develop type 2 diabetes is important for clinicians for rational preventive strategies. Recently parameters of low grade inflammation and non-alcoholic fatty liver were suggested as simple measurements to be included in the set of diabetes prognostic parameters. We therefore analysed the prognostic value of white blood cell count (WBC) and alanine aminotransferase (ALT) for conversion of IGT to type 2 diabetes.
Materials and Methods: The STOP-NIDDM trial is a multinational prospective study in subjects with impaired glucose tolerance (IGT). This report analysed the participants of the ITT analysis (n=1,368), 682 were randomized to acarbose treatment and 686 to placebo. Median follow-up time was 3.0 years. Included were subjects aged 40–70 years with fasting plasma glucose (FPG) levels >= 5.6 mmol/L and IGT by WHO definition. Age and parameters of metabolic syndrome according to NCEP III criteria were collected. Diabetes was diagnosed by 75g OGTT. WBC and ALT were measured by routine laboratory methods. WBC>=6.2 G/L and ALT >=40.4/31.3 U/L (male/female) were considered to be elevated. The Hazard ratio (HR) was calculated by Cox regression analysis adjusted for treatment and stratified by country.

Results: Two hundred eigthy five (42%) subjects in the placebo group and 221 (32%) in the acarbose group converted to diabetes. The mean baseline level of WBC was 6.33 (SD 1.65) G/L and for ALT 31.70 (21.14) U/L. Only subjects with increased WBC levels had an elevated risk for diabetes (HR 1.44; p<0.001), but not subjects with elevated ALT levels (HR 1.10; p=0.36). In multivariate analysis with metabolic syndrome (NCEP III), treatment group, FPG, 2 hPG, HbA1C, triglycerides and WBC were independent predictors of newly diagnosed type 2 diabetes.
Conclusion: WBC but not ALT is a simple and routinely measured laboratory parameter which may be also used for risk estimation of developing diabetes. However, ALT could not be confirmed as independent risk factor in subjects with IGT.
Support: Research grant by Bayer AG

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Effects of lifestyle modification on metabolic parameters and carotid intima-media thickness in patients with type 2 diabetes
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Background and Aims: Lifestyle modification is known to have positive effects on glycemic control and cardiovascular risk factors. Diabetes is a risk factor for cardiovascular disease. The carotid intima-media thickness (IMT) is considered to be an index of the progression of atherosclerosis. The aim of this study was to evaluate the effect of a 6 month lifestyle modification intervention on metabolic parameters and carotid IMT in patients with type 2 diabetes.
Materials and Methods: Sixty five patients with type 2 diabetes were randomly assigned into 2 groups, the lifestyle modification (LSM) group and the control (CON) group. The patients in the LSM group attended an intensive lifestyle modification intervention program for 16 weeks and had monthly meetings after the program. Patients in the CON group had no change in their usual treatment. Fasting plasma glucose, 2 hour postprandial glucose, HbA1c, lipid profiles, hsCRP, fasting insulin level, carotid IMT, blood pressure, and body indices were measured at baseline and after 6 months.
Results: LSM group showed a significant reduction in HbA1c (-0.98 ± 1.22 vs. +0.05 ± 1.24%, p=0.002), fasting plasma glucose (-28.72 ± 26.44 vs. +6.15 ± 44.91 mg/dl, p=0.022), and 2 hour postprandial glucose (–37.63 ± 44.79 vs. +14.77 ± 80.12 mg/dl, p=0.003) after 6 months. Total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, $HOMA_{IR}$, and hsCRP levels showed no significant difference. Body weight (–2.01 ± 2.59 vs. +0.22 ± 1.73 kg, p=0.001), BMI (–0.80 ± 1.00 vs. +0.02 ± 0.80 kg/m^2, p=0.003), systolic blood pressure (–8.15 ± 15.92 vs. +0.42 ± 14.07 mmHg, p=0.041) were significantly decreased in the LSM group. Significant carotid IMT regression was seen in the LSM group after 6 months (mean IMT: –0.050 ± 0.144 vs. +0.083 ± 0.167 mm, maximum IMT: –0.084 ± 0.197 vs. +0.07 ± 0.199 mm, p=0.004, p=0.009, respectively).
Conclusion: Lifestyle modification in patients with type 2 diabetes had positive effects on glycemic control, weight loss, and prevention of carotid atherosclerosis progression.

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The role of renin-angiotensin system blockers in the prevention of new -onset diabetes: meta-analysis of randomized controlled trials
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Background and Aims: Diabetes is associated with significant morbidity and mortality, especially when it is associated with cardiovascular disease. Prevention of diabetes will have significant impact especially among high risk patients.
Materials and Methods: We searched The Cochrane Controlled Trials Register (CCTR), MEDLINE, and EMBASE in January 2005. We included Randomized Controlled Trials (RCTs), in which outcomes of New -Onset Diabetes was reported. No language restrictions were applied. All identified trials were reviewed independently by two reviewers to determine whether trials should be included or excluded.
Results: Ten studies met inclusion criteria, including 77,541 participants. There was a statistically significant reduction in the incidence of New -Onset Diabetes in patients receiving Renin-Angiotensin System Blockers (ACEI or ARB) compared to other antihypertensive agents (RR 0.79; 95% CI 0.75–0.84, ARR=-0.02, NNT=50). There was a statistically significant reduction in the incidence of New-Onset Diabetes in patients receiving Renin-Angiotensin System Blockers compared to Diuretics, Conventional antihypertensive therapy (Diuretics or B-Blockers), and Calcium Channel Blockers (NNT=90,153, 62, respectively). There was a statistically significant reduction in the incidence of New-Onset Diabetes in patients receiving ACEI compared to Diuretics, and Conventional antihypertensive therapy. There was a statistically significant reduction in the incidence of New-Onset Diabetes in patients receiving ARB compared to other antihypertensive agents.
Conclusion: Renin-Angiotensin System Blockers significantly prevent the development of New-Onset Diabetes and should be utilized especially among high risk patients like patients with metabolic syndrome.

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Effects of a community-based intervention programme promoting physical activity on risk factors for diabetes and cardiovascular disease
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Background and Aims: Physical inactivity is an important risk factor for type 2 diabetes. Lifestyle intervention programmes focusing on diet and physical activity have documented that type 2 diabetes can be prevented in high-risk groups, but this approach is insufficient to stem the world wide epidemic of obesity and diabetes. Community-based strategies addressing the population at large are highly requested. We therefore designed an intervention study promoting physical activity on a district level in Oslo.
Materials and Methods: We selected the population in a low-income, urban district with high mortality rates and high prevalence of diabetes, obesity and physical inactivity for the intervention and the population in a neighbour district for controls. Baseline investigation of 2950 participants, 30–67 years old, follow-up investigation of 1776 (67% of those eligible). Of these 56% were women, 18% non-western immigrants. A set of theory-driven, low-cost intervention activities aimed at promoting physical activity was implemented in the intervention district from 2000 to 2003, tailored towards groups with different psychosocial readiness for change in physical activity. We used communication activities, invited to training sessions, labelled walking trails and offered a low-threshold fitness-test twice yearly. Main outcome measures were net changes observed between district cohorts in self-reported physical activity, psycho-social mediators related to physical activity, daily smoking, body weight, systolic blood pressure and serum levels of glucose and lipids.
Results: The net increase in physical activity as measured by two self-reported instruments was 9.5% (p=0.008) and 8.1% (p=0.02), and psychosocial mediators for change improved more (MANOVA, combined vector; P=0.002) in the intervention compared to the control district. The net proportion quitting smoking was 2.9% (95% confidence interval 0.1% to 4.0%, P= 0.043). The weight gain was less in the intervention district, with a mean net difference of 1.2 kg (0.6 to 1.9, P=<0.001) in men and 0.3 kg (–0.4 to 0.9) in women. Beneficial effects were also seen for the levels of triglycerides 0.16 mmol/l (0.06 to 0.25, P=0.002), cholesterol/HDL-cholesterol ratio 0.12 (0.03 to 0.20, P=0.007) and systolic blood pressure 3.6 mm (2.2 to 4.8, P<0.001), and in men also for the serum level of glucose 0.35 (0.03–0.67) mmol/l.
Conclusion: A community-based, low-cost intervention programme resulted in significant increase in physical activity in the intervention district followed by beneficial changes in several risk factors for diabetes and cardiovascular disease.
Support: The Norwegian Research Council

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Prenatal stress: effects on energy homeostasis and cognitive performance
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Background and Aims: The metabolic syndrome is a growing health problem in western industrialized societies. Whereas environmental factors, such as stress and diet, can propagate the aetiology of the metabolic syndrome during adulthood, one hypothesis states that they can also do so during the perinatal stage. For instance, exposure to stress during gestation is known to result in physiological, behavioral, and perhaps cognitive deteriorations during adulthood that finally lead to obesity and glucose intoler-

ance in aged rats. The changes in these parameters during young adulthood are largely unknown. Therefore, we investigated the influence of prenatal stress on energy balance, glucose homeostasis and cognitive performance in rats between 5 and 7 months of age.
Materials and Methods: Pregnant Wistar rats were subjected to immunological stress (LPS injections), psychological stress (chronic mild stress, CMS), or no stress at all. Body weight of the male offspring was measured regularly, and at age of 5 months, these rats were surgically equipped with jugular vein cannulas for blood sampling. To assess leptin sensitivity, the effect of a bolus leptin injection (25 ug iv) on food intake was measured. Intravenous glucose tolerance tests (IVGTT) and insulin sensitivity tests (IST) were performed to examine glucose homeostasis. After this, cognitive performance was tested in a novel object recognition test and an active shock avoidance test.
Results: CMS resulted in heavier male offspring, whereas LPS had no effect on offspring body weight. Neither CMS nor LPS caused major disturbing effects on glucose homeostasis. In fact, the CMS animals appeared to be more glucose tolerant during an intravenous glucose infusion. As opposed to the seemingly similar regulation of substrate homeostasis, CMS as well as LPS offspring performed less well in the "Novel Object Recognition" test (i. e., a standard cognitive performance task) relative to controls.
Conclusion: We conclude that perinatal stress differentially affects cognitive performance and regulation of energy balance in young adult offspring. Provided that perinatal stress leads to metabolic disregulation during ageing, the results in the present study demonstrate that metabolic disregulations are not required for the effects of prenatal stress on cognitive performance.
This study was supported by a career development grant from the dutch Diabetes Fonds to Dr. G. van Dijk on aetiology of the insulin resistance syndrome.

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Improved metabolic outcome and delayed structural rebuilding of pancreatic islets after regular intake of milk derivate in incipient diabetic Zucker rats (fa/fa)
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Background and Aims: Healthy nutrition is of key importance in preventing or even treating type 2 diabetes mellitus (T2DM). In this respect, the beneficial effects of probiotics have increasingly attracted public attention. The influence of a regular daily intake of a milk derivate (SMP) on the metabolic outcome and the progress of T2DM in Zucker rats (ZR, fa/fa) was studied.
Materials and Methods: Glucose-intolerante male fa/fa Zucker rats 15 weeks old were given daily either SMP in tap water ad libitum (25 g/l, SMP-group) or tap water alone(CO-group). Body weight, water and food intake and morning non-fasting blood glucose (BG) was determined weekly. Before, 3 and 6 weeks after treatment insulin, triglycerides and OGTT were monitored and 24 h blood glucose profiles were determined. At the end of the study pancreata were examined by immunohistochemistry and morphometry.
Results: SMP declined BG increase (5 weeks treatment: 6,1 ± 1,2 mmol/l vs. 7,5 ± 1,1 mmol/l in CO; $p<0,05$) and improved glucose tolerance (area under the curve, G-AUC after 6 weeks: 569 ± 145 vs. 780 ± 138 mmol x min/l; $p<0,05$). The insulin-AUC was increased with SMP (I-AUC: SMP=897 ± 120 vs. CO=745 ± 506 ng x min/l). The G-AUC correlated in SMP treated animals negatively with the portion of insulin producing cells in pancreatic islets ($r= -0,666$, $p< 0,05$), which was increased in SMP vs. CO (61,1 ± 4,7 vs. 51,9 ± 3,9%).
Conclusion: Daily intake of SMP in Zucker rats reduced insulin resistance, improved glucose tolerance and prevented structural rebuilding of pancreatic islets. This opens new avenues in the prophylaxis and / or treatment of metabolic syndrome and type 2 diabetes. The experimental results should be verified in clinical studies.

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Regulation of weight and obesity

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Mutation analysis of small heterodimer partner (SHP, NROB2) gene among 596 Chinese subjects and identification of four novel variants
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Background and Aims: The atypical orphan nuclear receptor small heterodimer partner (SHP, NROB2) modulates the transcription activity of MODY1 gene HNF-4α. Mutations in SHP were associated with moderate obesity among Japanese. The purpose of the study was to evaluate the prevalence of SHP variants among obese Chinese men.
Materials and Methods: We screened the whole coding region and intron/exon boundaries for SHP in 324 unrelated Chinese obese subjects (BMI 27.8 ± 2.7, BMI ≥25 kg/m^2 is the cut off for obesity in this study) and 272 unrelated nondiabetic and nonobese control subjects (BMI 20.3 ± 2.5, BMI <23 kg/m^2) by direct sequencing of the amplified polymerase chain reaction products.
Results: We identified six variants in 324 Chinese obese subjects, which included the previously reported mutations (H53fsdel10, R34X) in Japanese obese subjects. The H53fsdel10 was identified in seven separate obese carriers(2.2%) and R34X was identified in one carriers in this study. Additionally, a total of four novel mutations, including two missense mutations (G174A and G192E), one silent mutation (P10P) and one variants in intron1 (IVS1+10 C→T) were identified. The G174A and G192E variants were each identified in two separate obese carriers and P10P was identified in one carrier, the IVS1+10 C→T variants was also identified in one carrier. The overall frequency of the SHP mutations in Chinese obese objects in this study was 3.7% (12/324). All the mutations present in the heterozygous state. No mutations were identified in 272 nondiabetic lean controls ($P=0.00068$). Although, it was previously well documented that H53fsdel10 and R34X mutation were associated with obesity among Japanese, whether the four novel mutations have any functional significance needs further investigation.
Conclusion: Genetic variation in the SHP gene may be a key genetic factor responsible for moderate obesity among Chinese.
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Genetic interaction between the *IGF2* ApaI polymorphism and the insulin variable number of tandem repeats in their associations with body mass index in children
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Background and Aims: The insulin (*INS*) gene variable number of tandem repeats (VNTR) and single nucleotide polymorphisms (SNPs) in the nearby *IGF2* gene on chromosome 11 have been reported to be associated with weight gain. We therefore sought association between these genetic variants and weight at age 7 years in a normal population of children and explored possible gene-gene interactions.
Materials and Methods: Genomic DNA was extracted from blood and mouthwash samples from 1,400 children in the prospective Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC) birth cohort (the Children in Focus and control sub-cohorts). Of these 621 also had microsatellite-validated DNA samples from both their parents for transmission disequilibrium testing. Plasma IGF-2 concentrations were measured at age 5 years and heights and weights were measured at age 7 years. Body mass index (BMI) was calculated as weight (kg) divided by height (m)^2, and was converted into a standard deviation score (SDS) by reference to the full ALSPAC cohort (n~14,000). All samples were genotyped for *IGF2* ApaI (rs680; G+820A) and XcmI (rs3842759; A+6815T) (two *IGF2* SNPs reported to be associated with BMI in adults), and HphI (rs689) as a surrogate for *INS* VNTR class. Genotyping was performed by PCR followed by restriction fragment length polymorphism analyses.
Results: The *IGF2* XcmI SNP was associated with variation in plasma IGF-2 concentrations at age 5 years (geometric means: A/A 382 ng/ml, A/T 404, T/T 446; $p=0.001$) but not with markers of childhood obesity. In contrast both *IGF2* ApaI ($p<0.05$) and *INS* VNTR ($p<0.05$) were independently

associated with BMI SDS in children aged 7. There was also a significant interaction ($p<0.05$) between these genetic markers, with the largest BMI SDS being in those children who were homozygous class III for the *INS* VNTR and G/G for *IGF2* ApaI (Table). The association between variation in BMI SDS, *INS* and *IGF2* ApaI genotype could not be confirmed by parental transmission, neither was there a detectable parent-of-origin effect.
Conclusion: *IGF2* ApaI is associated with childhood BMI and this is partially independent of that associated with the nearby *INS* VNTR, suggesting these markers reflect two separate contributory sites. There is also a gene-gene interaction which might be mediated through altered *IGF2* expression, since both the *INS* VNTR and *IGF2* have previously been associated with variation in plasma IGF-2 concentrations and these with altered weight gain. Both the *INS* VNTR class III and *IGF2* ApaI genotype effects on body mass indices may reflect linkage disequilibrium with other causal variants rather than being directly causal themselves, especially since the genetic associations could not be confirmed by parental transmission.

Mean BMI SDS in healthy children aged 7 years, split by INS VNTR class and IGF2 ApaI genotype.

INS VNTR Class ———— IGF2 ApaI Genotype	I/I	I/III	III/III
A/A	–0.37	–0.31	+0.43
A/G	–0.01	–0.09	+0.21
G/G	+0.07	0.00	+0.65

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Association of a polymorphism within the protein kinase C beta promoter with adipositas
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Background and Aims: Adipositas is a high risk factor for a variety of diseases like type 2 diabetes mellitus (T2DM) and cardiovascular diseases. We investigated five single nucleotide polymorphisms (SNP) within the gene for the protein kinase Cβ (PRKCB1) regarding their influence on the risk for an enhanced body-weight or adipositas.
Materials and Methods: We investigated 583 caucasian individuals randomly collected in the region of Berlin and Potsdam, Germany. The cohort contained 317 controls, 94 subjects with T2DM, 165 subjects with IFG, IGT or both and 7 subjects with T1DM. The age was 51.8 ± 14.4a, the BMI 28,0 ± 5,8 kg/sqrm. The subjects were characterized by OGTT, anthropometric data and blood-parameters. The genotype of the SNPs was determined by the TaqMan technology. Promoter-assays were carried out by use of a promoter-fragment of the PKC beta cloned into the pGL3-basic vector (Promega) containing either the frequent or rare allele for the polymorphism.
Results: A polymorphisms lying in the promoter of PKC beta at the position -546 (C/G) was found to be associated with an increased risk for adipositas. Regarding all subjects of the cohort, homozygous carriers of the rare allele (GG) showed highly significant increased values for their body-weight (10%), BMI (10%, 30.6 ± 6.3 compared to 27.6 ± 5.6), waist-circumference (8%) and hip-circumference (7%) compared to homozygous carriers of the frequent allele. Even heterozygous individuals (CG) showed slightly increased values. Also, regarding only controls the GG-genotype was significantly associated with increased body-weight (11%), BMI (10%), waist-circumference (11%) and hip-circumference (6%). Interestingly, individuals carrying the GG-genotype tend to decreased fasting adiponectin levels. Promoter-assays with a PKC beta-promoter-luciferase construct containing either the C- or the G-allele revealed a decreased promoter-activity for the G-allele.
Conclusion: We conclude that the GG-genotype of the single nucleotide polymorphism at position -546 in the PKC beta-promoter represents a risk-factor for adipositas, since carriers of the GG-genotype reveal a mean BMI of above 30 kg/sqrm. A mechanism for this increased body-weight might be an altered promoter-activity of the PKC beta-promoter caused by the polymorphism.

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The association between the Pro12Ala polymorphism in the PPAR-gamma 2 gene and obesity depends on the level of physical activity
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Background and Aims: Previous studies have shown a more consistent association between *PPARG* Pro12Ala and obesity in women than in men. Results in intervention studies with both physical activity and diet have shown that the development of obesity differed between the genotypes in *PPARG*. The Skaraborg Project in Vara, a small community in Southwestern Sweden, was set up to study the development of obesity, type 2 diabetes, and hypertension with emphasis on the interaction between genetics and life-styles. In this population we explored the interaction between genotypes defined by the Pro12Ala polymorphism and leisure time physical activity in the association with obesity in men and women separately.
Materials and Methods: Between 2001 and 2003 a random sample of men and women aged 30–74 years in the population of Vara was invited for a survey of cardiovascular disease risk factors. In all 1811 subjects were included (81% participation rate). DNA was extracted from whole blood and this study was based on those 901 women, and 904 men with successful genotyping of Pro12Ala polymorphism in the peroxisome-proliferator-activator receptor gamma *PPARγ* -2 gene. The frequency distribution was in agreement with the Hardy-Weinberg expectations. As the Ala12Ala frequency was very low, the two genotypes containing Ala alleles were combined in all analyzes. Information (questionnaires and interviews) covered physical activity at work and at leisure time (LTPA), smoking habits, socio-economic background, and medical history. Specially trained study nurses ascertained anthropometric measures with standardized methods. Obesity was defined as a BMI ≥30 kg m^{-2}.
Results: Obesity was found in 25% of the women and 20% of the men with an increasing prevalence by age, especially in women. The proportion carrying the Pro12Ala polymorphism variant allele was 24% in both sexes. There was a strong inverse association between LTPA and obesity in both men and women (p for trend 0.001 and <0.001, respectively). The Ala allele was associated with obesity in women over 50 years (OR: 1.79, CI: 1.05–3.07). A stronger association with obesity was seen in women with a low LTPA (OR: 2.01, CI: 1.10–3.67), but the association was not present in those with a high level of LTPA (OR: 1.06, CI: 0.19–5.91). This association was not seen in men or in woman <50 years of age.
Conclusions: We found that the Ala allele of the *PPARG* Pro12Ala polymorphism was associated with obesity in women above 50 years. This risk was prevented already by a moderate level of LTPA. Considering the high prevalence of the Ala-allele there are implications for public health and prevention, and the findings support the emphasis on physical activity in the prevention of obesity.
This study was supported by grants from the Swedish Medical Research Council, the Skaraborg Institute, West Gothaland Region, the NEPI Foundation, Malmo University Hospital, Region Skane, Faculty of Medicine Lund University

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The PPARγ Pro12Ala polymorphism may influence the association of physical activity with lipid profile, insulin sensitivity and glycemic control in type 1 diabetes
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Background and aims: PPARγ is a transcription factor involved in the regulation of lipid metabolism and insulin signaling. The common Pro12Ala polymorphism of the gene has been associated with obesity, dyslipidemia, insulin resistance and risk of type 2 diabetes so that Ala-carriers have a lower risk. Physical activity (PA) has been shown to have a positive effect on lipid profile and insulin sensitivity in type 1 diabetes. However, the beneficial effect of PA show large inter-individual variation, a phenomenon that may in part be genetically determined. Therefore, we studied the association of the Pro12Ala-polymorphism on the effect of PA on lipid profile, insulin sensitivity and glycemic control in type 1 diabetes.
Material and methods: This is a cross-sectional study involving 346 type 1 diabetic patients (male/female:161/185, mean age 41.8 ± 10.1 years, dura-

tion of diabetes 38.3 ± 7.8 years) from the nationwide, multicenter Finnish Diabetic Nephropathy (FinnDiane) Study. Patients with end-stage renal disease were excluded. Genotyping was performed using TaqMan® technology. PA was assessed by a quantitative questionnaire and expressed in MET*h/week (MET=metabolic equivalent). Serum lipids and HbA_{1c} were measured by enzymatic methods. Insulin sensitivity was assessed by estimated glucose disposal rate (eGDR) with an equation developed by Williams et al 2000. Associations were evaluated by linear correlations.
Results: Pearson´s correlation coefficients with log-transformed PA according to genotype.

	All patients (N=346)	*Pro12Pro (N=237)*	*Pro12Ala (N=101)+ Ala12Ala (N=8)*
Total cholesterol	–0.085	–0.015	–0.250**
HDL-cholesterol	0.074	0.033	0.146
LDL-cholesterol	–0.062	0.016	–0.229*
Log Triglycerides	–0.108*	–0.032	–0.265**
Log eGDR	0.221**	0.174*	–0.347**
HbA_{1c}	–0.107*	–0.061	–0.205*

*P<0.05, **P<0.01

Conclusions: These preliminary data suggest that the Pro12Ala polymorphism of the PPARγ gene might influence the association between physical activity and lipid profile, insulin sensitivity and glycemic control in type 1 diabetes.

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Overexpression of adiponectin targeted to adipose tissue in transgenic mice
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Background and Aims: Adiponectin (ApN) is an adipokine, whose expression and plasma levels are inversely related to obesity and insulin-resistant states. Chronic repercussions of ApN treatment or overexpression on adiposity and body weight are still controversial. In this study, we investigated the chronic effects of a local, additional but homotopic expression of ApN *in vivo*.
Materials and Methods: We generated a heterozygous transgenic (Tg) mouse line with overexpression of ApN targeted to adipose tissue (native full-length ApN being placed under the control of the adipocyte aP2 promoter). Tg mice and wild-type (WT) littermates were maintained on a high-sucrose or high-fat diet for 5 months. On several occasions, body weight was mesured and tail vein blood collected from fed animals for determination of glucose, plasma ApN and triglycerides (TG). Some mice also underwent an oral glucose tolerance test (OGTT) or an insulin tolerance test (ITT) after an overnight fast. Adipose tissue from different depots (inguinal, gonadal, retrovesical) were collected at the end of the study. Adipose tissue mRNA levels were mesured by real time RT-PCR and circulating ApN levels by RIA.
Results: When compared to WT littermates, ApN mRNA levels were increased by 2- to 3- fold in every fat depot of Tg males or females, whatever the diet administered (p<0.05 or less). Plasma ApN levels were 2- to 10- fold higher in Tg than in WT mice (p<0.05). Body weight of Tg mice was slightly reduced (–4 to –12%, p<0.05 or less) while food consumption was unaltered or even slightly elevated. Accordingly, weights of the different fat depots were reduced by up to ~ 60%. In the basal state, fed blood glucose and plasma TG levels were decreased in Tg mice. During the OGTT, blood glucose and insulin levels were lower in Tg than in WT mice. The ITT confirmed the enhanced insulin sensitivity. Reduced adiposity of Tg mice could be explained by the increased expression of uncoupling protein 2 (UCP2), involved in energy dissipation and the diminished expression of fatty synthase, a key enzyme involved in lipogenesis, in adipose tissue. Abundance of TNFα mRNA, which is implicated in insulino-resistance, was also decreased in fat tissue of Tg mice.
Conclusion: Chronic overexpression of ApN targeted to adipose tissue led to reduced adiposity in spite of preserved calorie intake. Concomitantly, insulin sensitivity and the TG profile were improved. Low fatness may result from local upregulation of UCP2 and downregulation of FAS. This work may open new perspectives for the management of obesity and related metabolic diseases.
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The importance of catch-up growth after early malnutrition for the programming of obesity in adult
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Background and Aims: Low birth weight due to fetal growth retardation is associated with increased risk of developing glucose intolerance, type-2 diabetes, hypertension and cardio-vascular disease in adult life. It is less clear how the fetal and postnatal environment might predispose to obesity. We investigated whether a catch-up growth after maternal malnutrition should favour the development of obesity at adulthood in rat.
Materials and Methods: Dams were submitted to protein (8% vs 20%) or calorie (50% vs ad libitum) restriction during gestation. During lactation, half of the protein-restricted pups were maintained on the low protein diet whereas the other half as well as the calorie-restricted pups were nursed by mother fed the control diet ad libitum. A catch up growth was induced in these two latter groups by reducing the number of pups nursed by dams. At weaning, rats were transferred to laboratory chow and half of each group received, in addition, a supplement rich in fat and sucrose to induce obesity. Body weight, daily energy intake, plasma glucose, insulin and triglycerides, glucose tolerance, adipocyte cellularity, and leptin were measured, as well as the expression in fat tissue, of factors involved in risk to cardiovascular disease (Plasminogen Activator Inhibitor-1 -PAI-1-, angiotensinogen, adiponectin).
Results: Protein and calorie restriction during gestation led to growth retardation at birth. Permanent growth retardation appeared if malnutrition was prolonged throughout lactation. However, offspring overfed during the suckling period presented a rapid catch-up growth and became heavier than controls. The effect was enhanced with time and at the age of 10 months, the restricted offspring were heavier than controls (p<0.05). Administration of the hypercaloric diet induced obesity in every group and was associated with hyperleptinemia, hyperglycemia, hyperinsulinemia and glucose intolerance. In rats submitted to food restriction until weaning, the development of obesity was similar to that observed in controls. In contrast, rats having presented a catch up growth after birth developed a higher propensity for obesity compared to animals fed normally during early life (p<0.05 for protein-restricted rats, p<0.01 for calorie-restricted animals). This was not due to a modification of appetite regulation since the food intake was similar in all obese groups. Obesity was associated with an increased PAI-1 mRNA expression in control animals, but not in growth-restricted rats. It was associated also to a reduction in angiotensinogen and adiponectin expression, without effect of early malnutrition.
Conclusion: Catch-up growth immediately after early malnutrition should be a key point for the programming of obesity.

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Does initial leptin concentration influence the efficacy of weight reduction in patients with simple obesity?
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Background and Aims: The aim of the study was to evaluate the relation between the initial serum leptin and insulin level and the efficacy of non-pharmacological intervention in simple obesity.
Materials and Methods: The study group comprised 96 simply obese patients (53 women, 43 men) of Department of Internal Medicine, Metabolic Disorders and Hypertension, who underwent antiobesity treatment. Mean age was 47,6 ± 8,8 years, mean BMI - 38,2 ± 5,7 kg/m^2. Relative change of body mass (%Δ) was calculated as a ratio of complete change to the initial result. Considering the effect of a year - long weight reduction program, patients were divided into four groups: no effect, weight loss of 0–5%, weight loss of 5–10%, weight loss of >10%. Fasting serum concentrations of leptin and insulin were evaluated by radioimmunoassay.

Results: The initial leptin concentration and the effect on non-pharmacological treatment:

	Leptin	SD	Insulin 0	SD	Insulin 2	SD
No weight loss	68,59	28,04	36,6	10,3	82,2	24,2
0–5% loss	38,21	22,42	23,4	12,7	47,6	22,8
5–10% loss	35,02	13,23	14,7	6,4	29,1	9,9
>10% loss	32,07	27,25	9,6	2,7	20,5	5,6
P	<0,05		<0,05		<0,05	

In multivariate regression analysis considering % body mass reduction as a dependent variable, and the initial fasting serum insulin, leptin levels, and body weight as non-dependent variables only insulin concentration was an independent predictor of body weight reduction (BETA =0,47, R^2 =0,25).
Conclusion: High initial concentrations of insulin, but not leptin, coexist with low efficacy of antiobesity treatment.

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Neuronal activation of the human cerebral cortex by administration of insulin detemir compared to human insulin

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Background and Aims: Magnetoencephalography (MEG) has recently been introduced as a method to study neuronal activity in the cerebral cortex during insulin treatment. We have shown that in lean individuals, insulin increases the beta activity calculated from the magnetoencephalograms, whereas in obese individuals, this effect of insulin appears to be lost. Insulin detemir, a new basal insulin analogue has been associated with less weight gain compared to NPH insulin during insulin treatment. We therefore hypothesised that insulin detemir may affect beta activity to a different extent than human insulin.
Materials and Methods: We studied neuronal activity of the cerebral cortex using MEG in 10 overweight (BMI 29 ± 1 kg/m^2, HbA1c 5.7 ± 0.1%) non-diabetic human subjects during 1) a 2-step hyperinsulinemic euglycemic clamp (each step 90 min) with insulin detemir (D) (1st step 0.5 mU/kg/min, 2nd step 2 mU/kg/min) or 2) human insulin (I) (1st step 0.25 mU/kg/min, 2nd step 1 mU/kg/min) and 3) saline infusion (S). MEG recordings were performed at basal conditions, in the first and in the second step of D, I and S. Change of cortical activity during I and D (relative to S) were considered to be a measure of cortical action of I and D. Therefore, beta activity in I and D was divided by beta activity in S at each dose level.
Results: For I, the relative beta activity was 1.01 ± 0.05, 1.05 ± 0.05, 1.01 ± 0.03 at basal, 1st step and 2nd step respectively (p=0.80). However, after D administration beta activity increased (basal 0.98 ± 0.09, 1st step 1.09 ± 0.06, 2nd step 1.19 ± 0.09, p=0.04) with a significant time*condition interaction between I and D (MANOVA p=0.008). Moreover, the mean glucose infusion rate (GIR) was lower under D (1st step 9 ± 1 µmol/kg/min, 2nd step 26 ± 3 µmol/kg/min) than under I (1st step 11 ± 1 µmol/kg/min, p=0.01; 2nd step 36 ± 3 µmol/kg/min, p=0.003).
Conclusion: In conclusion, insulin detemir increased MEG measured cortical activity in overweight human subjects. In contrast, human insulin had no effect, confirming previous findings that in obese individuals the insulin effect on beta activity appears to be lost. Moreover, the effect of insulin detemir was similar to the previously measured effect of human insulin in normal non-obese individuals. Therefore, a restoration of the cortical insulin effect might be implicated in the beneficial effect of insulin detemir on body weight development.
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Women with type 2 diabetes have a higher calculated absolute CVD risk than men; the Hoorn-Diabetes Care Study

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Background and Aims: Treatment guidelines advise a strict control of prevailing risk factors to lower the increased cardiovascular risk in type 2 diabetes patients. Surprisingly, data of the absolute CVD risk in large diabetes populations are scarce. In contrast we know from epidemiological studies that the relative increased risk in female diabetes patients is about double that of men. The aim of our study was to assess the absolute cardiovascular risk (ACR) in patients with type 2 diabetes, estimated with the Oxford Risk Engine (UKPDS 56) separately for men and women.
Materials and Methods: Data from the managed diabetes care system in the Hoorn region of the Netherlands was used. Information on diabetes duration, blood pressure, HbA1c, lipid levels, and medication was available from 3480 subjects [1692 men, aged 59,6, and 1788 women, aged 62,2 years] who had their annual review visit in 2004. Furthermore, we assessed whether the use of blood pressure and lipid lowering medication was different for men and women.
Results: The mean calculated 10 year ACR was 14,3% (95% CI: 13.8–14.8) and 26.6% (95% CI: 25.7–27.5) for men and women, respectively. Cardiovascular risk factors (mean (CI)) differed significantly for men and women with respect to systolic blood pressure: 145 (144–146) vs. 151 (150–152); and total cholesterol: 4.9 (4.85–4.95) vs. 5.3 (5.25–5.35) for men and women, respectively. Lipid-lowering medication was significantly less prescribed to women than men: 39% and 44%, respectively. The use of blood pressure medication was higher in women: 70.2% vs. 62.1%, respectively. Also women received more different anti-hypertensive drugs.
Conclusion: In conclusion, in a dedicated managed care system the absolute cardiovascular risk is higher for female than for male patients. Partly this may be explained by lower statin use and by a more difficult to treat hypertension, requiring more drug use. Therapeutic strategies need to be developed which facilitate adequate cardiovascular risk management especially for female diabetes patients.

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Obesity and exercise

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Postprandial substrate oxidation in obese individuals with and without type 2 diabetes

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Background and Aims: Substrate oxidation abnormalities have been observed in obese non diabetic and diabetic subjects during OGTT. The extent to which substrate oxidation is altered after a mixed meal is, however, unclear particularly in type 2 diabetic patients. The aim of the study was to evaluate carbohydrate (CHO) and fat oxidation after a fat rich meal in diabetic and non diabetic obese subjects compared to normal weight controls.
Materials and Methods: Six obese type 2 diabetic, six obese non diabetic and six normal weight control male subjects, aged between 30 and 60 years, with normal fasting lipid levels, participated in the study. Insulin sensitivity was evaluated by euglycemic hyperinsulinemic clamp and, on a different day, substrate oxidation, measured by indirect calorimetry, and plasma metabolic variables were evaluated at fasting and for 6 hrs after a standard fat rich meal (944 kcal, 57% fat, 31% CHO, 12% proteins). Body composition was measured by BIA.
Results: Obese subjects with diabetes and without diabetes had similar insulin-resistance (M value= 3.9 ± 0.3 and 4.5 ± 0.6 mg/kg b.w./min) (M±SEM), compared to controls (7.1 ± 0.5 mg/kg b.w./min; p<0.001). Fasting and postprandial plasma insulin levels were higher in the two groups of obese compared to the controls, but 2 hrs after the meal plasma insulin was higher in the obese non diabetic than in the obese diabetic subjects. Fasting carbohydrate and fat oxidation rates were similar in the two groups of obese and in the controls. In the postprandial period the incremental area of carbohydrate oxidation decreased and that of fat oxidation increased in diabetic patients (CHO: -3.66 ± 1.32 g/kg fat-free mass (FFM)•6 h; Fat: 4.16 ± 1.20 g/kg FFM•6 h) compared to obese non diabetic subjects (CHO: 7.6 ± 3.5 g/kg FFM•6 h, p<0.02; Fat: -2.67 ± 1.2 g/kg FFM•6 h, p<0.003) and controls (CHO:1.7 ± 1.9 g/kg FFM•6 h, p<0.04; Fat: 0.66 ± 1.17 g/kg FFM•6 h, p=0.06). No significant difference in postprandial carbohydrate oxidation was observed between obese without diabetes and controls. However, obese subjects showed a trend toward a lower postprandial fat oxidation compared to controls (p=0.08).
Conclusion: These preliminary data suggest that obese diabetic patients oxidize more fat than carbohydrate in the postprandial period compared to normal-weight controls as well as to non diabetic obese subjects with similar insulin-resistance but higher postprandial early insulin response.

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Platelet resistance to nitric oxide donors, prostacyclin and anti-aggregating cyclic nucleotides in obese subjects is reverted by weight loss

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Background and Aims: Subjects with central obesity exhibit platelet hyperactivity, which is involved in the increased risk of developing atherosclerosis and cardiovascular events. Platelet alterations are at least in part attributable to impaired synthesis and/or action of the two anti-aggregating cyclic nucleotides guanosine 3',5'-cyclic monophosphate (cGMP) and adenosine 3',5'-cyclic monophosphate (cAMP), which are the mediators of the physiological platelet antagonists nitric oxide (NO) and prostacyclin. Aim of the present study is to evaluate whether platelet resistance to nitric oxide, prostacyclin, cGMP and cAMP can be reverted by weight loss obtained by diet intervention.
Materials and Methods: 20 obese subjects (M/F:9/11, age: 39.6 ± 1.9, BMI 34.7 ± 0.97 kg/m^2, waist circumference 102.9 ± 2.17 cm) were submitted to 6-month intervention program with diet alone to obtain a weight loss of at least 10% of initial weight. Platelet samples were obtained before and at the end of intervention. On each sample, we investigated by aggregation test (Born's method) the influence on platelet responses to ADP exerted by: i) the prostacyclin analog Iloprost (0.31-5 nmol/l) and the cAMP analog 8-bromo-cAMP (10–500 µmol/l); the NO donor sodium nitroprusside (SNP) (5–100 µmol/l) and the cGMP analog 8-bromo-cGMP (10–500 µmol/l). IC_{50} (minimal concentration of each inhibitor necessary to reduce platelet response to ADP by half) was determined.
Results: 10 subjects (4M/6F) reached the weight loss goal (body weight from 98.3 ± 3.46 kg to 84.7 ± 1.78 kg; BMI from 35.0 ± 1.46 to 30.3 ± 1.06; mean weight reduction: 13.3 ± 1.44%; waist circumference from 102.7 ± 3.49 to 92.8 ± 2.43; HOMA-IR index from 7.8 ± 1.21 to 3.3 ± 0.43) (Group A) and 10 subjects failed to reach the target (5M/5F; BMI from 34.4 ± 1.34 to 35.4 ± 1.32; HOMA-IR index from 5.5 ± 0.68 to 6.8 ± 0.94) (Group B).
All the subjects included in group A exhibited after the weight loss a significant increase of platelet sensitivity to antiaggregating agents as evidenced by a lower IC_{50} for SNP (8.2 ± 0.5 vs 23.5 ± 2.7 µ mol/l, p=0.0001) and for Iloprost (0.73 ± 0.09 vs 1.71 ± 0.24 nmol/l, p=0.0001). When parameters before and after weight loss were pooled together, IC_{50} of Iloprost and SNP correlated with body weight (r=0.575; p=0.008 and r=0.721, p=0.0001, respectively), BMI (r=0.788; p=0.0001 and r=0.566, p=0.0009, respectively), waist circumference (r=0.524; p=0.018 and r=0.681, p=0.0001, respectively) and HOMA index (r=0.654; p=0.002 and r=0.638, p=0.002, respectively). Also the anti-aggregating effect of 8-Br-cGMP and 8-Br-cAMP was increased after weight loss. Subjects of study B did not show any significant increase in platelet sensitivity to antiaggregating mediators after 6-month intervention: IC_{50} for SNP being 22.4 ± 1.3 vs 25.6 ± 3.6 µ mol/l (ns) and IC_{50} for Iloprost being 1.68 ± 0.27 vs 1.78 ± 0.26 nmol/l (ns).
Conclusion: Platelet resistance to the anti-aggregating effects of NO, prostacyclin and cyclic nucleotides in central obesity is reversible after about 10% weight loss with diet alone. Weight loss is therefore an important intervention measure to reduce the increased prothrombotic risk in obesity.

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Exenatide (exendin-4) reduced HbA_{1c} and weight over 82 weeks in overweight patients with type 2 diabetes

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Background and Aims: Many therapies for type 2 diabetes mellitus (DM2) are associated with weight gain. This post-hoc analysis examined HbA_{1c} and weight effects of exenatide, an incretin mimetic, in patients with DM2 who were unable to achieve glycemic control with metformin (MET), a sulfonylurea (SFU) or MET+SFU, and who completed 82 weeks of treatment.
Materials and Methods: The 82-week completer cohort comprised 393 patients who completed one of three 30-week, placebo (PBO)-controlled trials and 52 weeks in the subsequent open-label extensions: 62% male, age 56 ± 10y, HbA_{1c} 8.3 ± 1.0%, BMI 34 ± 6 kg/m^2 (mean±SD). During the 30-week PBO-controlled trials, subjects were randomized to PBO (n=128), or 5 µg (n=128) or 10 µg (n=137) exenatide administered subcutaneously BID, with a 4-week 5 µg exenatide BID dose-initiation period for the 10 µg arm. Subsequently, subjects continued in open-label extensions in which all subjects received 5 µg exenatide BID for 4 weeks, followed by 10 µg exenatide BID. Subjects continued MET, SFU, or MET+SFU.
Results: At Week 30, exenatide treatment resulted in reductions from baseline in both HbA_{1c} (PBO: +0.1 ± 0.1%, 5 µg: -0.9 ± 0.1%, 10 µg: -1.1 ± 0.1% [mean±SE]) and body weight (PBO: -0.6 ± 0.3 kg, 5 µg: -1.9 ± 0.3 kg, 10 µg: -2.7 ± 0.4 kg). Effects on HbA_{1c} and weight for subjects treated with 10 µg exenatide BID for 82 weeks were sustained with reductions from baseline in HbA_{1c} of –1.1 ± 0.1% (95% CI: –1.3 to –0.9%) and progressive reductions in weight of -4.5 ± 0.5 kg (95% CI: –5.5 to –3.5 kg). At Week 82, subjects who received placebo from Weeks 0 to 30 prior to exenatide had reductions in HbA_{1c} of -1.2 ± 0.1% (95% CI: -1.4 to -1.1%) and weight -3.3 ± 0.4 kg (95% CI: -4.0 to -2.5 kg) from their Week 30 extension baseline. The percent of subjects with baseline HbA_{1c} >7% who achieved HbA_{1c} ≤7% at Weeks 30 and 82 was 48% and 51%, respectively, for subjects receiving 10 µg exenatide BID for 82 weeks (n=128). The most frequent adverse events were generally mild to moderate nausea and vomiting, hypoglycemia, and diarrhea.
Conclusion: Reductions in both HbA_{1c} and body weight with exenatide treatment were durable over 82 weeks.

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TeleObe: Telemedical support programme for long-term treatment of obese children and adolescents

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Background and Aims: In children and adolescents overweight and obesity are associated with increased carotid intima media thickness and high risks for the development of glucose intolerance and insulin resistance followed by type 2 diabetes. It was the goal of the project to develop an effective strategy not only to reduce body weight, but also to induce a long-term stabilisation of body weight with the result of minimizing metabolic and cardiovascular risk factors.
Materials and Methods: Totally 75 patients (age 14.1 ± 2.3 ys, 38 females [50.7%]) were recruited to participate in the first phase of the trial. In the second phase the telemedical support programme TeleObe was started. It integrates data collection, visualization, and recommendations for handling by using mobile phone and Internet services. Its core is a module, which visualizes a summary of the patients' clinical data of the last months. A feedback from the physician to the patient is given periodically. At the end of the trial a questionnaire on patients experiences and satisfaction is applied.
Results: In the first phase of the project, during a period of 34.1 ± 6.9 days the children and adolescents participated in a specially designed structured treatment and teaching programme. It integrates medical advices, psychological treatment aiming a modification in eating habits, leisure time, physical exercise and sports. During this period patients showed a reduction in body-mass index (BMI) from 30.5 ± 5.0 to 27.9 ± 4.6 kg/m^2 ($p<0{,}001$). Following this first phase the telemedical support programme was started: Still, a high percentage of 92% of children and adolescents initially recruited, participated in the follow-up. During the follow-up further modifications were registered in respect of eating habits in 88% of the patients and physical exercise and sports in 67%. Parallel, in the mean there was still a tendency to further reduction of body weight (BMI 26.2 ± 3.7 kg/m^2, $p=0.079$). Using the telemedical support programme motivational and psychological problems were identified in 29% of the patients followed by feedback and specific intervention from physicians, psychologists or educators specialized for questions and training in physical exercise, sports and/or eating habits. All in all about 90% of participants considered telemedical support helpful for the everyday management and to improve empowerment and motivation.
Conclusion: In Western industry countries about 10 to 20% of children and adolescents suffer from obesity with excessive risk for diabetes and cardiovascular diseases, for example indicated by increased carotid intima media thickness. Still, the number of patients is dramatically increasing. The telemedical support programme TeleObe proved to be feasible and highly effective. It integrates not only monitoring of body weight control but allows via feedback the identification and specific intervention in motivational, psychological or other problems in everyday management. It is highly accepted by obese children and adolescents and seems to be an effective tool also for routine management in long-term treatment.
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Effects of chronic physical training on phenotype characteristics, insulin sensitivity and malonyl CoA regulation in subjects with type 2 diabetes

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Background and Aims: A sedentary lifestyle is associated with insulin resistance and an increased risk of type 2 diabetes. It has been postulated that an increased concentration of malonyl CoA in muscle constitutes a main molecular mechanism behind this relation.
Materials and Methods: We have investigated AMP-activated protein kinase (AMPK) - malonyl CoA – malonyl CoA decarboxylase (MCD) – axis in muscle as well as insulin sensitivity, substrate utilisation and regional fat distribution in12 nearly normal-weight and well-controlled patients with type 2 diabetes (HbA1c 5.5 ± 0.3%). They were studied before and after a 12-week moderate-intensity exercise training programme. Post exercise biopsies were taken 24–36 h after last bout of exercise when the euglycemic-hyprinsulinemic clamps were performed. Basal and insulin-stimulated concentrations of malonyl CoA, as well as amounts and activities of AMPK and acetyl-CoA carboxylase (ACC), and malonyl CoA decarboxylase (MCD) were determined in biopsies from the vastus lateralis muscle.
Results: Training increased glucose infusion rate (M value) by 77% (3.0 ± 0.5 vs 5.3 ± 0.9 mg/kg/min, $p=002$), which was associated with a 15% decrease in intraabdominal fat area (145 ± 15 to 123 ± 14 cm^2, $p=0.02$). Neither the phosphorylation of AMPK (Thr 172) nor of acetyl CoA carboxylase (ACC) in muscle were increased after exercise training. The basal concentration of malonyl CoA in muscle, however, was decreased by 28% (0.18 ± 0.01 to 0.13 ± 0.01 nmol/g, $p=0.004$), which was accompanied by an increase in activity of MCD by 23% (0.65 ± 0.02 to 0.80 ± 0.02 nmol/min/mg, $p<0.01$), and a decrease in diacylglycerol (DAG) levels by 46% (60 ± 8.3 to 41 ± 3.3 nmol/g, $p=0.02$).
Conclusion: Long-term moderate-intensity exercise training markedly improved insulin sensitivity and decreased intraabdominal fat in nearly normal-weight patients with type 2 diabetes. The decrease of malonyl CoA concentration in muscle and decrease of DAG levels improved fuel sensing and insulin sensitivity after exercise training. We propose that the increased activity of MCD plays a crucial role in this context.

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Effects of community-based resistance training on glycaemic control maintenance in persons with type 2 diabetes

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Background and Aims: Several trials conducted in the laboratory setting have demonstrated that progressive resistance training (PRT) can improve glycaemic control in patients with type 2 diabetes. However, it is unclear whether improved glycaemic control can be maintained through PRT performed in the community setting. We have investigated whether, for adults with type 2 diabetes, a maintenance enhancement intervention involving community-based or home-based PRT could sustain the improved glycaemic control resulting from laboratory-based training.
Materials and Methods: We studied 57 overweight (BMI ≥27) sedentary men and women aged 40–80 years with established (>6 mo) type 2 diabetes. Initially, all participants attended a twice weekly 2-month supervised PRT program conducted in our exercise laboratory. Thereafter, participants were randomised to PRT maintenance programs (2 times per week) in either the: (1) community fitness and recreation centre (Centre), or (2) the home (one hand weight, minimal instruction) (Home) for 12 months. Participants also attended monthly lifestyle education seminars throughout the 12 month maintenance period. Glycaemic control (HbA_{1c}) and body composition (bioimpedence) measurements were collected at baseline, 2-months, and 14-months.
Results: The mean exercise adherence during the initial 2-month program was 82.9 (sd: 22.3)%. Compared to baseline, when all participants were pooled together mean HbA_{1c} changed by -0.4% (95% CI: -0.6 to -0.2), lean mass increased by 0.7 kg (0.2 to 1.2) and there was no change in fat mass at the completion of the initial 8 week program. The 12-month maintenance program was completed by 27 of the 28 participants randomised to the Centre group and 26 of the 29 of the Home group. The mean exercise adherence was 65.8% (27.3) and 62.8% (30.5) for the Centre and Home groups respectively. Within-group comparisons showed that HbA_{1c} remained lower than baseline values at 14-months in the Centre group [-0.4% (-0.7 to -0.03)], but not the Home group [-0.1% (-0.4 to 0.3)]. However, there were no between-group differences for the changes in HbA_{1c}, fat mass and lean mass during the maintenance period (2–14 months). Changes in HbA_{1c} during the maintenance period were inversely correlated with exercise adherence (-0.42; $P = 0.02$) in the Centre group only.
Conclusion: In adults with type 2 diabetes, a 12-month PRT program undertaken in a community fitness and recreation centre achieved a small improvement in glycaemic control over baseline values, but home training did not. The low exercise adherence probably accounted for the limited benefits. Behavioural methods to improve adherence to prescribed exercise need to be developed and tested in future trials.
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Risk analysis for decrease in activities of daily living and physical activity in the elderly patients with type 2 diabetes mellitus using 4-year prospective follow-up study

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Background and Aims: The final treatment goal for the elderly patients with diabetes mellitus is to maintain their quality of life(QOL). Many studies demonstrated that decreases in activities of daily living(ADL) and physical activity deteriorate QOL of the elderly. The aim of the present study was to investigate risk factors for decrease in ADL and physical activity in the elderly patients with type 2 diabetes mellitus using 4-year prospective follow-up study.
Materials and Methods: Subjects were 130 elderly(age:65 year old or more, mean age±SD:72 ± 6) outpatients with type 2 diabetes, visited our Medical Center for 4 years. Patients with serious visual disturbance, serious other diseases and/or dementia were excluded from the study. Subjects were consisted of 70 males and 60 females. Duration of diabetes, BMI, HbA1c and modality of treatment(diet alone: oral hypoglycemic agent: insulin) were 13 ± 9 year, 24 ± 3 kg/m^2, 6.9 ± 1.0% and 21%:63%:16%, respectively. Frequencies of subjects with complications at the baseline were as follow: diabetic retinopathy: 36%, nephropathy: 46%, neuropathy: 61%, hypertension: 46%, hyperlipidemia: 25%, and atherosclerotic disease: 27%. ADL score and physical activity including working, leisure activity and sports, were measured before and after 2- and 4- year follow-up by the Tokyo Metropolitan Institute of Gerontology index of competence for elderly people and Beacke index, respectively. Effects of 18 clinical factors on changes in ADL and physical activity were examined biennially using chi-square, monovariate and multivariate regression analysis.
Results: (1)ADL score and leisure activity score at 4 year were decreased ($p<0.05$) in comparison with each score at the baseline. (2)Monovariate analysis revealed that decrease in ADL score at 2 year was negatively related to ADL score ($p<0.01$) and total cholesterol levels ($p<0.05$) at the baseline, and that decrease in ADL score at 4 year was positively related to age ($p<0.02$) and triglyceride levels ($p<0.01$) at the baseline and development and/or progression of diabetic retinopathy ($p<0.05$) and atherosclerotic disease ($p<0.01$) and negatively related to total and HDL cholesterol levels ($p<0.02$) at the baseline. Decrease in total score of physical activity at 2 year was positively related to HbA1c at the baseline ($p<0.05$). (3) Multivariate analysis revealed that decrease in ADL score at 2 year significantly related to decreased ADL score at the baseline and diabetic retinopathy ($p=0.0003$), and that at 4 year to higher age and serum creatinine levels and diabetic retinopathy ($p<0.0001$). On the other hand, decrease in total score of physical activity at 2 and 4 year significantly related to decreased physical activity at the baseline ($p=0.0002$). In the model excluded physical activity score at the baseline, another significant factor for decrease in physical activity was HbA1c levels ($p<0.05$).
Conclusion: Risk factors for decrease in ADL were higher age, lower ADL, dyslipidemia and development and/or progression of diabetic vascular complication. On the other hand, risk factors for decrease in physical activity were physical activity itself and HbA1c. These results suggested that encouragement to increase physical activity including leisure activity, as well as metabolic control, might be important to maintain QOL in the elderly patients with type 2 diabetes mellitus.

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Exercise capacity in patients with type 1 diabetes mellitus under euglycaemic and hyperglycaemic conditions

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Background and Aims: Circumstantial evidence suggests that subjects with type 1 Diabetes mellitus (DM) tend to perform physical exercise under higher blood glucose levels than recommended for prevention of long-term complications. It is, however, unknown whether an increase in plasma glucose availability improves exercise capacity. Therefore, physical exercise capacity was assessed in a eu- and hyperglycaemic condition in subjects with type 1 DM in a randomised single-blinded cross-over trial.
Materials and Methods: Well-trained subjects with type 1 DM on continuous subcutaneous insulin infusion (CSII) were included. Eight male subjects (age 37.9 ± 12.5 years, mean±SD; diabetes duration 20.1 ± 9.2 years) volunteered for the trial. Baseline individual exercise capacity was assessed on a cycle ergometer (mean $VO2_{max}$ 44.9 ± 7.9 ml/kg). Glycemic control, food intake, and training practice were standardised before each test. Subjects were randomly allocated to perform two stepwise ergometer tests in a eu- or hyperglycaemic condition with at least 7 days between tests. Subjects were blinded to the clamped glucose levels and to the achieved exercise capacity. All tests started at a level corresponding to 20% of the individual maximum power according to the baseline test. Power was increased to 40% and 60% after 10 and 20 minutes, respectively. After 30 minutes power levels were augmented every 30 seconds by 10 watts until physical exhaustion. The primary endpoint was the peak power output (PPO), secondary endpoints were the rate of perceived exertion (RPE, assessed on Borg Scale), lactate levels (LL), heart rate (HR), and respiratory exchange ratio (RER). A Conjugate Bayesian Analysis for mean effects (CBA) was performed to investigate the robustness of our results.
Results: Eu- and hyperglycaemic clamp conditions were at a plasma glucose concentration of 5.3 ± 0.6 mmol/l and 12.4 ± 2.1 mmol/l, respectively. Glucose concentrations remained constant throughout the test and differed significantly between tests ($p<0.0001$) with a difference of 7.1 ± 2.1 mmol/l. Hyperglycaemia did not result in a significant increase in PPO compared to euglycaemia (271.3 ± 45.9 Watts vs. 276.3 ± 39.4 Watts; $p=0.49$). Hyperglycaemia did not significantly impact on the secondary endpoints (RPE, LL, HR and RER) compared to euglycaemia. Sensitivity analyses by means of a CBA confirmed our results.
Conclusion: In subjects with type 1 DM exercise capacity is not influenced by hyperglycaemia. Therefore, hyperglycaemia cannot be recommended for physical activity. Comparable LL and RER suggest that an increase in extracellular glucose availability did not translate into increased intracellular glucose oxidation.
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Exercise training protects the renal circulation against high glucose challenge

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Background and Aims: We have previously shown that high glucose levels, in the range observed in the post-prandial period in patients with type 2 diabetes, cause direct and acute endothelial dysfunction in the non-diabetic isolated perfused rabbit kidney. Exercise training is considered to have beneficial effects in cardiovascular diseases involving endothelial dysfunction, including DM2, most likely through an increase in vascular shear stress and consequent increase in endothelial-dependent vasodilation. This study assessed whether exercise training is able to maintain normal renal vascular endothelial function despite high glucose exposure.
Materials and Methods: Non-diabetic rabbits were pen confined (SED) or treadmill trained over a 12-week period of running for 5 days a week at a speed of 18 m/min during 60 min at no incline (0%) (ExT). Isolated kidneys were acutely perfused ex vivo (3 h) with normal (5.5 mM – control group) or high (15 mM) D-glucose, the latter corresponding to 2 h post-breakfast median [272.5 mg/dl (15 mM)] values obtained from a cohort of 780 Brazilian type 2 diabetic outpatients regularly attending the diabetes clinic at State University of Rio de Janeiro. The renal circulation was sub-maximally pre-contracted with NE (0.5 µM), before testing the relaxing effects of increasing cumulative concentrations of the endothelium-dependent vasodilator acetylcholine (ACh).
Results: In the SED 5.5 group, ACh induced dose-related vasodilator responses, reaching the maximum of 41 ± 2% ($n = 10$; $P < 0.05$). In the kidneys perfused with high concentrations of glucose (SED 15), endothelium-dependent vasodilation was significantly blunted. Maximal relaxation in the presence of 15 mM glucose was of 19 ± 2%, which was significantly different from the SED 5.5 group (41 ± 2%, $n = 10$, $P < 0.01$). In the ExT 5.5 group, ACh-induced vasodilation was significantly enhanced when compared to the SED 5.5 group, reaching the maximum of (52 ± 2%, $n = 10$, $P < 0.05$). The exposure of the renal circulation of ExT animals to high glucose did not change endothelium-dependent vasodilation induced by ACh (46 ± 3%, $n = 6$), when compared to the ExT 5.5 group. Moreover, exercise training prevented the deleterious effects of high glucose on endothelial-dependent renal vasodilation (SED 15: 19 ± 2% vs. ExT 15: 46 ± 3%; $P < 0.05$).
Conclusion: Exercise training protects the rabbit renal circulation against endothelial dysfunction elicited by acute exposure to moderately elevated glucose levels, corresponding to the postprandial glycemia of diabetes type 2 patients under treatment. The enhanced renal vasodilator reserve elicited by exercise training turns out to be a response that protects the kidney from the deleterious effects of glycemic peaks.

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Obesity and cardiovascular risk factors in patients with type 2 diabetes: data from the Swedish National Diabetes Register

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Background and Aims: The prevalence of obesity is increasing worldwide and may influence the course of type 2 diabetes. Pharmacological treatment of hyperglycemia reduces the risk for complications in patients with diabetes but is often accompanied by a weight gain that may increase the prevalence of cardiovascular risk factors. Here we wanted to study the effects of obesity and weight change on major cardiovascular risk factors in type 2 diabetic patients representing a large sample receiving routine care.
Materials and Methods: A cross sectional study was performed in 44.042 type 2 diabetic patients included in the Swedish National Diabetes Register in 2003. Data from 4.468 patients were available for a prospective analysis from 1997 through 2003.
Results: Obese subjects (36.9% of all subjects in the cross sectional analysis) had higher HbA_{1c}, blood pressure, and triglyceride levels, and lower HDL cholesterol compared to normal weight and overweight patients ($p<0.001$ for all comparisons), and high frequencies of hypertension, hyperlipidemia and microalbuminuria (88, 81, and 29%, respectively). BMI was a predictor of all of these variables both in the cross sectional study ($p<0.001$) and in the prospective study (BMI in 1997 predictive of variables in 2003; $p<0.01$–0.001), independent of age, sex, diabetes duration, smoking and type of hypoglycemic treatment. Obesity *per se* was associated with an increased prevalence of hypertension (OR 2.14 [2.01–2.27]), hyperlipidemia (OR 1.83 [1.74–1.93]), and microalbuminuria (OR 1.51 [1.43–1.59]) in the cross sectional analysis. Being obese in 1997 was associated with an increased risk of developing hypertension (OR 2.34 [1.90–2.90]), hyperlipidemia (OR 2.24 [1.94–2.59]), and microalbuminuria (OR 1.71 [1.47–1.98]) in 2003 in the prospective analysis, but there were no differences in follow-up HbA_{1c}-levels between who were normal weight, overweight or obese in 1997. The change in BMI during the six years of observation was independently associated both with change in HbA_{1c}, and change in blood pressure in all subjects. When analysed separately these associations were significant in patients on diet alone or oral hypoglycemic drugs (OHD) alone but chiefly absent in those requiring insulin for metabolic control.
Conclusion: The high frequency of hypertension, hyperlipidemia, and microalbuminuria in obese patients with type 2 diabetes implies an increased risk for cardiovascular disease and the need for therapeutic measures. Weight loss is associated with significant improvements in the control of several of these risk factors but the association is largely restricted to patients who are treated with diet or OHD alone. Thus, our results underline the importance of considering the influence of obesity and weight gain on cardiovascular risk factors in diabetes care but also suggest that there may be important differences between treatment categories.

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Clinical diabetes monitoring

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Is the frequency of self blood glucose measurements (SMBG) related to longterm metabolic control? Multicentre analysis including 24500 patients from 191 centres in Germany and Austria

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Background and Aims: Blood glucose measurements and insulin dose adjustments are generally accepted components of modern diabetes self management. However, few studies address the efficacy of SMBG under real-life conditions.
Materials and Methods: The DPV-Wiss-database is a standardized, prospective, multicentre, computer-based documentation of diabetes care and outcome. By March 2005, anonymized records from 24500 patients were selected from the database classified either as type-1 (n= 19491, mean duration of diabetes: 5.8 years, mean HbA1c 8.5%) or type-2 diabetes (n= 5009, mean duration of diabetes: 10.3 years, mean HbA1c: 7.9%), with concomitant documentation of antidiabetic therapy (insulin, OAD, diet), average number of blood glucose values recorded per week (SMBG), and metabolic control. For each patient, the most recent complete year of diabetes care was evaluated. Based on local reference ranges, HbA1c values were mathematically adjusted to the DCCT reference. The SAS 9.1 statistical software package was used for data analysis, applying multivariate regression models.
Results: On average, patients with type-1-diabetes performed 4.4 BG measurements per day. This number increased continuously during the last 10 years (1995: 3.1 values/day, 2004: 4.9, $p<0.0001$). In type-1 diabetes, after correction for age, gender, diabetes duration, insulin therapy and centre difference, the SMBG frequency was significantly associated with better metabolic control ($p<0.0001$). One additional daily BG measurement improved HbA1c by 0.26%. This relationship was present for paediatric and adult patients as well as for male and female patients. HbA1c reduction with higher frequency of SMBG was significantly more pronounced in patients on intensified therapy (4 or more daily injections or CSII: HbA1c reduction per additional measurement per day: 0.32%) compared to patients on conventional therapy (1–3 daily insulin injections: HbA1c reduction: 0.16%; $p<0.0001$). The average number of SMBG was significantly lower in type-2 patients (2.7 measurements/day). In 2021 patients with type 2 diabetes on insulin therapy, again more frequent SMBG was associated with better metabolic control (HbA1c reduction: 0.16% for one additional SMBG per day, $p<0.0001$), while in 2988 patients on OAD or diet alone (2.0 BG measurements per day), more frequent blood glucose measurements were associated with higher HbA1c levels (HbA1c increase: 0.14% for one additional SMBG per day, $p<0.0001$).
Conclusion: More frequent measurements of blood glucose are associated with better metabolic control both for patients with type-1 diabetes and for patients with type-2 diabetes on insulin. Dose adjustments by patients based on SMBG results are likely to explain this effect. In contrast, in type-2 diabetes on oral agents or diet alone, SMBG may be primarily recommended for those patients with suboptimal metabolic control.

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Self-monitoring of blood glucose in type 2 diabetes is associated with improved long-term outcome

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Background and Aims: The impact of self-monitoring of blood glucose (SMBG) on disease-related morbidity and mortality in patients with type 2 diabetes mellitus is unknown. We report a German multicenter, retrospective cohort study (ROSSO) addressing this issue.

Materials and Methods: In total, 3268 patients who had been diagnosed with type 2 diabetes between 1995 and 1999 were followed retrospectively with a cut-off date of end 2003. Endpoints were diabetes-related morbidity (a combined endpoint that included non-fatal myocardial infarction, stroke, foot amputation, blindness, and hemodialysis) and all-cause mortality.
Results: Mean follow-up time (±SD) was 6.5 (± 1.6) years. 1543 patients (47.2%) performed SMBG for at least one year. A non-fatal endpoint was experienced by 293 patients (9.0%), and 120 patients (3.7%) died. The rate of non-fatal events was 10.4% for the cohort without SMBG vs. 7.2% with SMBG (P = 0.002), the respective rate of fatal events was 4.6% vs. 2.7% (P = 0.004). Kaplan-Meier analysis demonstrated superior survival for both endpoints for the patients with SMBG (P <0.001). Cox regression analysis identified SMBG as an independent predictor of morbidity and mortality with adjusted hazard ratios of 0.68 (P = 0.009, 95 percent confidence interval 0.51 to 0.91) and 0.49 (P = 0.003, 95 percent confidence interval 0.31 to 0.78), respectively. The beneficial effect of SMBG on survival for both endpoints remained significant even when patients on insulin were excluded from the analysis. Subanalyses revealed that SMBG was associated with an earlier iniitiation of pharmaceutical diabetes therapy.
Conclusion: SMBG is associated with decreased diabetes-related morbidity and all-cause mortality in patients with type 2 diabetes. SMBG appears to provide benefit for both insulin-treated patients and patients not receiving insulin therapy.
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Frequency of self monitoring of blood glucose in type 2 diabetes and glycaemic control in a Swedish primary care setting
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Background and Aims: The cost of test strips used for self monitoring of blood glucose (SMBG) has increased in recent years and is in Sweden now exceeding 60 million euro a year. The benefit of the testing has been questioned and the aim of this study was to explore the use of SMBG and its association with glycaemic control in patients with type 2 diabetes in primary care.
Materials and Methods: A cross-sectional observational study was conducted in 2003 at 18 primary health care centers in Sweden, where all known patients with diabetes mellitus were surveyed. Depending on whether test strips for SMBG had been prescribed within the last year, patients were categorised as users or non-users of SMBG. Glycaemic control was estimated by HBA1c. After exclusion of patients with type 1 diabetes and elderly patients living in nursing homes, 3299 men and 3196 women with type 2 diabetes remained for further analyses. A random sample of 896 patients (533 users and 363 non-users of SMBG) stratified for age, gender and therapy group were selected for further exploration of data from medical records. The users were also subjected to a telephone interview about their opinions and habits of SMBG.
Results: When adjusted for age and gender there were no differences in HbA1c between users (6.9%) and non-users (6.8%) of SMBG in patients treated with insulin (n=2014) or in patients treated with oral agents (n=2401), (6.3% in both groups). In patients treated with diet only (n=2080), users of SMBG had higher HbA1c compared to non-users (5.5% vs. 5.4%, p=0.002). Baseline characteristics of the 896 patients from the sample are presented in table 1. There were no differences in HbA1c, blood pressure, dyslipidemia, mikroalbuminuria, antihypertensive or lipidlowering medication and no difference in prevalence of ischemic heart disease or smoking status between users and non-users of SMBG. Among the 533 users of SMBG the consumption of test-strips varied from 0 to 42 test-strips during the week prior to investigation and no correlation was found between the frequency of tests and HbA1c. In a linear regression adjusted for age and gender there was no association between frequency of SMBG-tests and HbA1c in the different therapy groups, respectively (diet p=0.62, oral agents p=0.13, insulin p=0.57).
Conclusion: The use or non-use of SMBG, as well as the frequency of testing among users of SMBG, was not associated with improved glycaemic control in any therapy category of patients with type 2 diabetes in primary care. We conclude that the lack of association between the usage of SMBG and improved glycaemic control could not be explained by differences in age, gender, smoking status, diabetes related complications or concomitant medication between users and non-users of SMBG.

Table1. Characteristics of 896 patients with type 2 diabetes, users and non-users of SMBG

	Diet non-user n=133 mean	Diet user n=176 mean	p	Oral agents non-user n=117 mean	Oral agents user n=190 mean	p	Insulin non-user n=113 mean	Insulin user n=167 mean	p
Age	68.4	65.6	0.048	66.4	64.7	ns	68.3	67.0	ns
HbA1c	5.4	5.5	ns	6.4	6.3	ns	6.7	6.8	ns
Systolic blood pressure (mmHg)	142	141	ns	142	142	ns	142	139	ns
Diastolic bloodpressure (mmHg)	80	79	ns	79	78	ns	76	76	ns
S-cholesterol (mmol/l)	5.4	5.2	ns	5.2	5.2	ns	5.2	5.1	ns
Number of teststrips/week	0	3.2		0	4.0		0	6.6	
Gender men (%)	52.6	49.4	ns	51.3	48.9	ns	53.1	49.1	ns
Smoking (%)	16.4	21.1	ns	21.3	18.2	ns	16.9	18.6	ns
Ischemic heart disease (%)	21.1	11.4	ns	17.1	18.9	ns	28.3	26.3	ns
Microalbuminuria (%)	17.0	15.1	ns	24.2	18.8	ns	33.3	36.6	ns
Statins (%)	28.6	27.3	ns	36.2	43.7	ns	44.2	38.3	ns

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Detecting insulin resistance in patients by postprandial measurement of urinary glucose: Study of 306 patients undergoing the OGTT
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Background and Aims: According to the DECODE study, the need for early detection and treatment of postprandial hyperglycemia is important and has been stressed in clinical medicine in recent years. The results of the DECODA study targeting Asian diabetic patients was similar to the results derived in the DECODE study. Compared with the Caucasian type 2 diabetic patient, the Japanese diabetic is more prone to become insulin deficient. In clinical medicine, the clinician does not pay attention to the importance of measuring urinary glucose.
In this study, the usefulness of measuring postprandial urinary glucose was investigated by analyzing the relationship between insulin resistance and urinary glucose during the 75-g OGTT(oral glucose tolerance test).
Materials and Methods: Our clinic is a specialized clinic of diabetes mellitus located in Tokyo, Japan. Patients were selected by risk assessment, for example, family history and obesity (BMI ≥ 25 is the criteria for obesity in Japanese). A total of 306 patients were given OGTT and were classified according to the 1998 WHO Diabetic criteria, (Mean±SE age: 54.1 ± 1.0 years for men, 59.6 ± 0.9 years for women; BMI: 24.8 ± 0.3 for men, and 24.3 ± 0.3 for women). The percentage of patients with a FPG(fasting plasma glucose) level <110 mg/dL, 110–125 mg/dL, ≥126 mg/dL, relative to those judged to have diabetes mellitus by OGTT was calculated. Patients with a normal OGTT response were also classified into two groups and compared (either ≥180 mg/dL or <180 mg/dL after 1-hr). In addition, the relationship between insulin resistance (HOMA-R: homeostasis model assessment R) and BMI was investigated.
Results: Among the 306 patients, 160 had diabetes mellitus, 75 were IGT, and 71 were normal. Among the 160 subjects diagnosed as having diabetes, the FPG level was 110–125 mg/dL in 39 subjects (25%) and was <110 mg/dL in 13 subjects (8%). These results suggest that, the diagnosis of diabetes will be missed in 33% of patients when judged from the FPG alone. However, when 2-hr urinary glucose level ≥100 mg/dL was used as a screening crite-

ria, it was found to be elevated in 156 out of 160 patients (98%) who were suspected of having diabetes mellitus.
In 22 patients with a normal OGTT, also the 1-hour glucose level was higher than 180 mg/dL, these patients were considered to be at high risk for developing diabetes according to the Japanese Diabetic Society, 19(86%) of them were obese with a high HOMA-R, had a positive 2-hr urinary glucose ≥100 mg/dL.
Conclusion: At present, FPG level is the gold standard for screening of diabetes in current clinical practice. However, it has been pointed out that diabetes mellitus may often be overlooked when the diagnosis is based on FPG values alone. The results of our study suggest that it is possible to avoid missing most of these patients who are not diagnosed as diabetic, by screening with a postprandial urinary glucose. In addition to a better method of detection, reducing economic burden with earlier diagnosis of diabetes will have a significant positive impact on health care costs.

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1,5-anhydroglucitol (1,5-AG, Glycomark™) and postprandial hyperglycemia as measured by continuous glucose monitoring system (CGMS) in inadequately controlled patients with diabetes
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Background and Aims: Postprandial glucose is an independent risk factor for macrovascular complications. Serum 1,5-anhydroglucitol (1,5AG) is a marker that drops as serum glucose exceeds its renal threshold, ~180 mg/dL. It responds sensitively and rapidly to hyperglycemia and may be abnormal even in patients with hemoglobin A1c (HbA_1c) at goal. The objective of this study is to demonstrate the relationship between 1,5AG and postprandial hyperglycemia as reflected by continuous glucose monitoring system (CGMS) in suboptimally controlled patients with diabetes.
Materials and Methods: Patients age 18 to 75 with type 1 or type 2 diabetes and an HbA_1c between 6.5 and 8% with stable glycemic control were recruited from two sites. Patients with severe comorbidities were excluded. A CGMS monitor was worn for two consecutive 72-hour periods and patients checked 7-point fingerstick glucose profiles. Area under the curve for glucose above 180 mg/dL (AUC-180) and mean glucose as determined by CGMS over each 72 hour period were compared to 1,5AG, fructosamine (FA) and HbA_1c at baseline, day 4 and day 7. Correlation coefficients and multivariate analysis of above relationships were explored. Data on the first 33 patients is included.
Results: 1,5AG varied considerably between patients (mean 6.5+/–3.2ug/mL) despite similar HbA_1c (mean 7.3+/–0.5%), suggesting more robust relationships with glucose dynamics. Both mean (r -0.45, p=0.006) and study end 1,5AG (r -0.46 p=0.008) correlated with AUC-180 with greater significance than did mean A1C (r 0.33, p=0.057), study end HbA_1c (r 0.32, p=0.072), mean FA (r 0.38, p=0.88) or study end FA, (r 0.30, p=0.088). Other cut-offs for AUC showed similar correlation. Mean (r 0.38, p=0.01) and study end HbA_1c (r 0.33, p=0.03) correlated significantly with mean glucose, but not 1,5AG or FA. To more fully evaluate the effect of postprandial glucose control on these analytes, multivariate regression compared 4 independent variables (sum of maximum glucoses post-breakfast, -lunch, and -dinner, and 7-day AUC-180), to mean 1,5AG and to % change in 1,5AG over the second 72-hour interval. R-values for 1,5-AG, HbA_1c, FA, and % 1,5AG change were 0.58, 0.36, 0.36, and 0.82, respectively; there was no significant relationship to % change of HbA_1c and FA. In contrast, the multivariate regression of premeal average glucose for breakfast, lunch, dinner, and 7-day mean glucose) was evaluated and correlated as follows: 1,5AG (r=0.34), HbA_1c (r=0.57), and FA (r=0.51).
Conclusion: The 1,5AG level reflects postprandial hyperglycemia to a greater extent than HbA_1c and FA. HbA_1c and FA reflect premeal and average glucose levels to a greater extent than 1,5AG. Despite moderate overall glycemic control, significant postprandial hyperglycemia, as determined by CGMS, may exist. This is reflected by 1,5AG and change in 1,5AG, which may be used as complementary markers to HbA_1c for determining overall diabetes control.

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Correlation of HbA_{1c} values with fasting and post-prandial blood glucose according to the quality of the glycaemic control and the magnitude of daily blood glucose excursions
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Background and Aims: To investigate the relation of HbA_{1c} with fasting (FBG) and post-prandial (ppBG) blood glucose and also evaluate the effect of good and bad glycaemic control and of small or great glucose diurnal fluctuations on this relation.
Materials and Methods: We studied 85 patients with type 1 (23) or type 2 (62) diabetes, 44 males, 41 females, of mean age 55.5 ± 1.9 and diabetes duration 15.4 ± 4.1. They were followed for 3 months during which they performed self-monitoring of blood glucose (BG) 2–4 times daily, immediately before or 2 hours after a main meal. Thus, we finally evaluated approximately 22000 BG measurements, 25% of which were fasting (morning), 25% pre-meal and 50% post-prandial. HbA_{1c} was measured at the end of the three months period and correlations were done with the mean, the fasting and the post-prandial BG values, at various time intervals. Furthermore the same correlations were compared between patients with good and bad glycaemic control and patients with great and small diurnal fluctuations of BG.
Results: HbA_{1c} showed a significant correlation with the mean of all BG values over each of the three months (r-value 0.429, 0.474 and 0.474, $p<0.01$), over the last two months (r-value .498, $p<0.01$) and over the three months (r=.505, $p<0.001$) of follow up. Analyzing this correlation separately for FBG adjusting for ppBG and for ppBG adjusting for FBG (partial correlations) a significant correlation was found between HbA_{1c} and ppBG over each of the three months (r=.348, .455, .358) over the last two months (.391, $p<0.01$) and over the three months (r=.420, $p<0.001$) but not with FBG (r=.136, .164, .125, .240, .208, NS respectively). Further analyzing this relationship separately in patients with good ($HbA_{1c}\leq 7\%$) and bad ($HbA_{1c}>7\%$) glycaemic control we found that, in the group with good control HbA_{1c} correlated with ppBG (r=0.318, $p<0.05$) but not FBG (0.222, NS), while in the group of worse control HbA_{1c} correlated with FBG (0.393, $p<0.05$) but not with ppBG (0.108, NS). Finally, we divided the patients in a group with great daily fluctuations of BG over the three months of follow-up (variability 42) and a group with small fluctuations (variability 20). Mean HbA_{1c} was not different between these two groups, 7.03vs7.04%. In those with small fluctuations HbA_{1c} correlated with both FBG (r=.449, $p<0.01$) and ppBG (r=.681, $p<0.01$) while in those with great fluctuations HbA_{1c} was not correlated either with FBG (r=.177, NS) or with ppBG (r=.290, NS), although the mean HbA_{1c} was not different between the two groups.
Conclusion: a) HbA_{1c} value depends more on post-prandial blood glucose, when glycaemic control is good and more on fasting blood glucose, when glycaemic control is worse b) HbA_{1c} is not representative of the quality of glycaemic control in patients with great diurnal fluctuations of the blood glucose c) the above should be considered in the evaluation of the diabetic patient in clinical practice.

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The diversity of phenotype between different FBG levels in the newly diagnosed type 2 diabetes with FBG lower than 7 mmol/l
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Background and Aims: In the early stages of type 2 diabetes, modest beta-cell lose in the face of persistent insulin resistance would result in increased postprandial glucose level represented by the loss of first-phase insulin release. However, the FPG is frequently lower than 7 mmol/L at that time. The elevating FPG manifests the metabolism of the patient is deteriorating. In this study we underline 143 patients (FPG lower than 7 mmol/l) and divided them into three groups by their fasting glucose levels to examine the diversity of metabolic disorder and insulin secretion.
Material:
Research subjects:
143 newly diagnosed type 2 diabetes (according to the 1999 WHO criteria) with FPG less than 7 mmol/L and average age 53.15 ± 12.63, males 81, females 62.
Groups:
Level 1: FPG<5.6 mmol/l,
Level 2: 5.6≤FPG<6.1 mmol/l
Level 3: 6.1≤FPG<6.9 mmol/l.

Methods:
(1) Anthropometric determinations: All subjects were measured height, weight, waist-to-hip ratio (W/H) and blood pressure
(2) Laboratory examination: OGTT (75-g oral glucose tolerance test), IRT (insulin releasing test), HbA1c and Blood lipid profiles.
(3) Insulin sensitive is determined by HOMA-IR, βcells function by HOMA-βcell, early secretion by Insulin release index (30 min Ins-0 min Ins / 30 min BG-0 min BG).
Statistical method: Data evaluation was conducted using the SPSS 10.0 grogram.
Results:
(1)The HOMA-βcell and Insulin release index are significant decreased as the FBG level increased.
(2)Paralleled with the FPG go up from level 1 to level 2 the insulin resistance is progressive. An elevated FPG from level 2 to level 3 indicate βcell dysfunction and worsening glycometabolism(HbA1c and P2 hPG ascend). (Tab 1)

Tab 1 phenotype diversity in different FPG levels

FPG	Level 1 <5.5	Level 2 5.6≤FPG <6.1	Level 3 6.1≤FPG <6.9	P value 1/2	2/3	1/3
N	49	41	53			
BMI (kg/m^2)	24.7 ± 3.31	25.3 ± 4.33	25.1 ± 2.72	>0.05	>0.05	>0.05
W/H	0.85 ± 0.06	0.87 ± 0.05	0.89 ± 0.05	>0.05	>0.05	>0.05
SBP(mmHg)	140.6 ± 18.1	141.3 ± 17.2	152.9 ± 17.8	>0.05	<0.05	<0.05
DBP(mmHg)	86.3 ± 12.1	85.5 ± 13.0	87.3 ± 12.9	>0.05	<0.01	<0.05
FPG (mmol/L)	4.65 ± 0.52	5.76 ± 0.21	6.45 ± 0.22	<0.05	<0.05	<0.01
P2 hPG) (mmol/L	11.38 ± 3.12	11.45 ± 3.79	12.34 ± 3.52	>0.05	>0.05	<0.05
FIns(mU/L)	15.84 ± 12.72	22.61 ± 15.82	14.56 ± 9.68	<0.01	<0.01	>0.05
P2 hIns (mU/L)	104.6 ± 60.59	114.5 ± 71.20	58.16 ± 42.97	>0.05	<0.01	<0.01
Cholesterol* (mmol/L)	4.65 ± 1.18	5.07 ± 1.64	4.9 ± 1.46	<0.05	>0.05	>0.05
Triglyceride* (mmol/L)	1.79 ± 1.58	2.45 ± 1.40	1.85 ± 0.81	<0.01	<0.01	>0.05
HDL* (mmol/L)	1.14 ± 0.25	1.26 ± 0.4	1.21 ± 0.34	>0.05	>0.05	>0.05
LDL* (mmol/L)	2.89 ± 0.86	2.93 ± 1.01	2.88 ± 1.26	>0.05	>0.05	>0.05
HbA1c(%)	6.56 ± 2.10	6.6 ± 0.81	7.6 ± 2.51	>0.05	<0.05	<0.05
MUA*(mg/L)	19.9 ± 7.34	24.6 ± 12.13	49.6 ± 22.91	<0.01	<0.01	<0.01
HOMA-IR*	2.82 ± 2.56	4.51 ± 3.36	4.19 ± 2.81	<0.01	<0.01	<0.01
HOMA-βcell*	341.83 ± 201.99	189.19 ± 125.71	98.15 ± 64.51	<0.01	<0.01	<0.01
$\Delta I_{30}/\Delta G_{30}$*	7.44 ± 7.24	6.50 ± 6.36	4.62 ± 3.43	<0.01	<0.01	<0.01

Data was expressed as mean±SD
*Profiles were log-transformed before analyses. Covariance analyses were conducted to adjust interaction factors. Metabolic parameters were compared by two-tailed t test or t' test, if necessary.
A P value of <0.05 was considered to be statically significant.

Conclusion: In the early stage of disease, the patients in different FPG levels have different phenotype diversity and show different metabolic disorders.

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The effect of a targeted intensive pharmacologic intervention on modifiable cardiovascular risk factors and coronary risk in patients with type 2 diabetes mellitus without clinical coronary artery disease
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Background and Aims: Patients with type 2 diabetes mellitus (T2DM) have the same coronary risk (CR) that non-diabetic subjects with previous events of coronary artery disease (CAD), due to the association between T2DM with cardiovascular risk factors (CRFs). Randomized trials have shown that a intensified intervention on CRFs has a beneficial effect to prevent CAD in T2DM patients, but is this a relevant effect in clinical practice? The Objective was to assess the effect of a targeted intensive pharmacologic intervention on CRFs and CR in patients with T2DM without CAD attended in our Endocrine Clinic.
Materials and Methods: In 482 T2DM patients without previous events of clinical CAD and one or more modifiable CRFs above recommended goals; HbA1C, fasting plasma glucose (FPG) cholesterol (Ch), HDL-Ch, triglycerides (TGs), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured, LDL-Ch (Friedewald) was calculated, ten years CR (Framingham) was predicted, and in 343 patients postprandial capillary glucose (PCG) was determined; before and after 1 year of pharmacologic therapy implementation. We applied the targets recommended by the European Diabetes Policy Group: HbA1C<6.5%, FPG<110 mg/dl, PCG <135 mg/dl, Ch <185 mg/dl, HDL-Ch >46 mg/dl, LDL-Ch <115 mg/dl, TGs <150 mg/dl, SBP <130 mmHg, DBP <80 mmHg and CR <20. The mean (±; S.D.) values for CRFs, the percent of patients who reached the targets, and the number of targets achieved were compared before and after 1 year of pharmacologic therapy implementation. For continous variables a paired-samples t test was used. For categorical variables a related samples MacNemar test was performed (SPSS for Windows, version 6.0).A level of P <0.05 was considered statistically significant.
Results: Mean (±; S.D.) age was 65.6 ±; 11.7 years, 13.6 ±; 10 years from diabetes diagnosis, and 42% were male.There was a decrease in all mean CRFs after intervention, being significant for HbA1C, FPG, PCG, Ch, HDL-Ch, LDL-Ch, SBP, DBP, and CR (P = 0.000 for all variables). The percentage of patients who reached the targets increased after intervention for all variables, except for HDL-Ch that decreased (P=0.0000), being significant for HbA1C, FPG, PCG, Ch, LDL-Ch, SBP, DPB and CR (P= 0.0000, = 0.01, = 0.006, = 0.0000, = 0.0008,= 0.0000,= 0.0000, =0.02 respectively).The number of targets achieved and the proportion of insulin treated patients and of subjects taking statins, ACEIs or ARBs, and antiagregants increased after intervention (P= 0.0000, = 0.0000, = 0.002, = 0.0000 respectively). Only 1 patient had an event of CAD during the period studied.
Conclusion: Our study indicate that, in clinical practice, a targeted intensive pharmacologic intervention on modifiable CRFs has a beneficial effect on predicted CR and to prevent primary vascular events in T2DM patients without previous clinical CAD.

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Analysis for atherosclerosis in type 2 diabetic patients using a novel pulse wave velocity measurement - cardio-ankle vascular index
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Background and Aims: In order to diagnose atherosclerosis in any part of the body, pulse wave velocity (PWV) measurement is a useful approach. Recently the classical method measures PWV of the brachial artery and the ankle (baPWV). It said that aortic PWV is not reflected though a large amount of past PWV measurements and baPWV is influenced by blood pressure. On the other hand, since the novel method, cardio-ankle vascular index (CAVI) is calculated from the electrocardiogram, phonocardiogram, brachial artery waveform and ankle artery waveform using a special algorithm, CAVI is not influenced by blood pressure. We analyzed risk factors for atherosclerosis in the patients with type 2 diabetes using CAVI.
Materials and Methods: The subjects of this study were 36 patients with type 2 diabetes (age 68 ± 9.1, 24 males and 2 females) and age matched 55 normal subjects for control. CAVI was measured using VaSera VS-1000 (Fukuda Electronics Co, Tokyo, Japan). Blood pressure, serum levels of triglyceride (TG), total cholesterol (TC), HbA1c and creatinine, and urinary albumin excretion (UAE), which were risk factors for atherosclerosis in patients with type 2 diabetes, was measured.
Results: The CAVI in type 2 diabetic patients was significantly higher than of normal subjects (8.63 ± 0.92 and 12.28 ± 3.37, respectively, p<0.01). There

was significant positive correlation between CAVI and age in normal subjects, however, no relationship between CAVI and age in type 2 diabetic patients was observed. In the patients with micro and macroalbuminuria, CAVI was 15.9 ± 3.3, which was significantly higher compared to those in the patients with normoalbuminuria (11.9 ± 3.6, p<0.01). CAVI of the diabetic patients with hypertriglycemia is significantly higher than that with normotriglycemia (10.9 ± 2.4 and 14.2 ± 3.7, respectively, p<0.001). In other parameter, blood pressure and serum levels of TC, HbA1c and creatinine, no significant differences were observed. In addition, a logistic regression analysis showed that mean systolic blood pressure and UAE were independent risk factors for increase in the levels of CAVI.
Conclusion: We report that the CAVI, a novel method for measurement of PWV, in type 2 diabetic patients was significantly higher than in the normal subjects. Triglyceride, blood pressure and UAE were risk factors for increase in the level of CAVI in the patients with type 2 diabetes. It is reported that CAVI is not influenced by blood pressure; therefore this new method represents a breakthrough in the diagnosis of atherosclerosis in diabetic patients.

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Use of HOMA-IR to assess the risk of incident diabetes among men with normal fasting blood sugar
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Aims: New diabetes screening recommendations recognize the importance of a rising blood sugar as a component of the metabolic syndrome and for the subsequent development of diabetes. However, even glucose thresholds set at 110 may miss a significant number of vulnerable subjects that could benefit from lifestyle interventions targeted at reducing the likelihood of developing diabetes. Since insulin levels rise prior to glucose derangements, we assessed the predictability of incident diabetes using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) among 971 individuals with normal glucose values.
Methods: 971 Participants from the Normative Aging study, a longitudinal follow-up study of aging in veterans, who provided both fasting glucose and insulin levels in 1986 and did not have a diagnosis of diabetes, were eligible for this study. Subjects were divided into two groups; those with a fasting blood sugar of < 110 or >= 110 mg/dl but less then 126 mg/dl. Within each group, HOMA-IR was divided into tertiles. We than determined the risk of developing type 2 diabetes (diabetes diagnosis and/or use of diabetes medication) using multivariate Cox proportional hazards.
Results: Approximately 10% of the cohort developed diabetes during a mean follow-up of 9 years. Among those with normal fasting glucose, higher HOMA values were predictive of diabetes (2nd and 3rd tertial RR 2.3 and 4.2 with test for trend P<0.0001), however among those with impaired fasting glucose HOMA was not predictive of diabetes over fasting glucose alone (test for trend p=0.37). Multivariate survival analysis confirmed the associations between time to diabetes and elevated HOMA values. For those with normal fasting glucose the relationship observed between HOMA and diabetes was significant (2nd and 3rd tertial RR 2.1 and 3.9 with test for trend P=0.0007). For those with impaired fasting glucose, HOMA was not additionally predictive of time to diabetes (p>0.2).
Conclusions: The identification of a pre-diabetic state among individuals with „normal" blood sugars could lead to earlier interventions to potentiality delay the onset of diabetes. Additional research is necessary to determine if measuring HOMA-IR in a clinical setting is both feasible and practical.

PS 59
Clinical diabetes outcome

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Control of glycemia and other risk factors in patients with diabetes. Results of medical intervention of the Polish 400 Cities Project
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Background and Aims: The Polish 400 Cities Project (P400CP) is a large scale interventional and educational program. The medical intervention of P400CP is aimed to increase detection and control of arterial hypertension, diabetes and lipid disorders in inhabitants of 418 small cities (<8 000 inhabitants) and adjoining villages, particularly in lower-educated people. The aim of the present study is to assess prevalence and control of cardiovascular risk factors in diabetic patients, who took part in screening tests in P400CP in years 2003 and 2004.
Materials and Methods: In 2003–2004 the medical intervention was performed in 123 cities. Altogether 38 976 subjects (age range 18–98 years; mean age: women (W) 57,1 ± 13,5; men (M) 56,6 ± 13,4) took part in screening tests. Complete data were obtained in 36 696 subjects (W, n=24 215; M, n=12 481) and analysed in the present study. During screening tests blood pressure (two readings during one visit), anthropometric measurements, laboratory tests (fasting glucose and total cholesterol, whole capillary blood by strip tests, Accutrend GCT) and questionnaire interviews were performed.
Results: During screening tests, 2 912 (7,9%) of examined subjects declared earlier detected diabetes (W 7,8%; M 8,2%; n.s.). In only 29,7% of them fasting glucose was <100 mg/dl (W 32,0%; M 25,5%; p<0,01). Mean age of subjects aware of diabetes was 64,7 ± 9,9 (W 65,5 ± 9,6; M 63,1 ± 10,3; p<0,01). Among diabetes subjects blood pressure ≥130/80 mmHg was observed in 95,4% (W 96,1%; M 94,1%; p<0,02). In 76,5% of diabetes subjects total cholesterol was ≥175 mg/dl (W 79,3%; M 71,3%; p<0,01). Overweight or obesity was observed in 87,4% of patients aware of diabetes (W 86,5%; M 89,2%; p<0,04). Their mean body mass index was 30,6 (SD 5,11) kg/m2 (W vs. M; n.s.). Abdominal obesity (W >88 cm, M >102 cm) was observed in 74,5% of diabetes subjects (W 82,4%; M 60,0%; p<0,01). Cigarettes were smoked by 6,1% of diabetes woman and 13,9% of men (p<0,01).
Conclusion: High prevalence and poor control of diabetes, and inadequate control of major cardiovascular risk factors in diabetes patients from small cities and villages in Poland call for urgent educational and preventive measures. The results of PP400M in years 2003–2004 strongly support decision of implementing this large scale project.

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The diabetes first 2004/2005 type 2 diabetes E-audit of meeting glycaemic and CHD risk factor targets in 126 Scottish GP practices
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Background: Elevated levels of blood glucose, blood pressure and cholesterol concentrations are associated with significant morbidity and mortality in patients with Type 2 diabetes. Accordingly, guidelines recommend aggressive targeting of all three risk factors to reduce the associated disease burden. We audited the proportion of patients meeting targets in 126 Scottish GP practices.
Method: Practices consented to regular data exports from their GPASS computer system in anonymised format to a central server. These data represent the latest exports for each practice, collected between 30/07/2004 and 01/03/2005. Patients were stratified by diabetes therapy category. Monotherapy patients represented the largest patient cohort, at an earlier stage of their disease and therefore the group with the greatest opportunity for reduction in long term complications with a target based approach to treatment. These patients were further stratified as to target attainment for HbA1c, blood pressure and cholesterol by guideline based targets and GMS audit standards. Criteria were defined as having been measured, if a result had been recorded in the 12 months prior to data export

Results: Audit results were available on 21083 patients. 19046 (90.3%) of patients had a measured HbA1c and, of those, 60.1% had an HbA1c >=7%. Patients taking monotherapy for glucose lowering represented the largest proportion of audited patients (35.9% of the total patient base), 91.3% of monotherapy patients had a measured HbA1c.
Conclusion: Amongst diabetes patients managed on oral monotherapy for Type 2 diabetes there is significant unmet healthcare need. Missed glycaemic control, blood pressure and cholesterol concentration targets are likely to impact significantly on morbidity and mortality in Type 2 diabetes. An aggressive, target based approach for these patients should be given strong consideration.

Breakdown of monotherapy results

	Patient number	Percentage of total measured
Monotherapy glucose lowering with HbA1c result	6901	
HbA1c>=7%	4089	59.3
HbA1c>7.4% (GMS target)	2754	40.0
Monotherapy glucose lowering with BP result	7007	
BP>=140/80	4354	62.1
BP>145/85 (GMS target)	2141	30.6
Monotherapy glucose lowering with cholesterol result	6397	
Patients with cholesterol>5.0 (GMS target)	1918	30.0

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Are type 2 diabetes management guidelines effective in daily practice? Results from the German LEADIT study
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Background and aims: This observational study evaluated whether evidence based treatment guidelines for type 2 diabetes, issued by the German Diabetes Association (DDG), are followed in daily practice and what effect treatment according to guidelines had on glycaemic control and cost effectiveness.
Materials and methods: Glycaemic efficacy was assessed by HbA1c-responder rates after 12 months. Responders were defined as patients reaching an HbA1c-goal < 7%. To evaluate the influence of promoting treatment guidelines in daily practice, an intervention group (LEADIT) was compared with a control group. In the LEADIT group, guidelines were actively promoted, the investigators were trained in guidelines and received special care throughout the study whereas in the control group there was no such training or care. At the beginning, all patients were treated with oral antidiabetic monotherapy and had an HbA1c >7.0%. Direct and indirect costs were analysed from the societal perspective and cost effectiveness was assessed as cost per responder.
Results: A total of 5892 patients were included. 3031 patients (2382 from LEADIT, 649 from the control group) were evaluable in terms of efficacy, safety and costs. At study start, the mean HbA1c was 8.0%. 1601 patients (53%) were treated according to guidelines and 1430 (47%) were not. Patients in the LEADIT group were more often treated according to guidelines (55%) than patients in the control group (43%). The HbA1c-responder rate in the LEADIT group (73.4%) was significantly higher ($p<0.0001$) than in the control group (57.9%). The mean treatment costs according to guidelines were comparable to non-guideline treatment (€ 1040 vs. € 1012 per patient and year). The cost effectiveness was better in patients treated according to guidelines (€ 1196 per responder vs € 1984).
Conclusions: In daily practice, treatment of patients with type 2 diabetes according to guidelines has significant advantages compared to non-guideline treatment in terms of glycaemic efficacy. The training of investigators had positive effects on the percentage of patients reaching the HbA1c-goal. Treatment according to guidelines did not lead to higher cost and was more cost effective than non-guideline therapy.

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Extent of glycemic control in treated patients with diabetes in a US managed care setting
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Background and Aims: Previous reports suggest that glycemic control remains suboptimal in spite of the availability and use of currently available antidiabetes treatment agents. This study examined the extent of glycemic control by treatment status for patients with diabetes within a US managed care setting.
Materials and Methods: The study population included 11,837 managed care patients enrolled from 6/1/2000 to 6/30/2001 within the Ochsner Clinic Foundation network, a large, non-profit, integrated healthcare delivery system. Diagnosis of diabetes was based on an inpatient or outpatient ICD-9 code for diabetes or a history of a pharmacy claim for any antidiabetes medication. Analyses examined the percentage of patients whose A1C level was below the current 2005 American Diabetes Association (ADA) A1C target (<7.0%), overall, and by whether they received antidiabetes medication(s) during the study period. The most recent A1C value during the study period was used. Mean distance from A1C treatment target was calculated for patients who received one or ≥ 2 classes of antidiabetes treatment during the study period. Medication classes included biguanides, meglitinides, sulfonylureas, thiazolidinediones, and insulin.
Results: Mean age of the study population was 63 years; 49% were male. Among 8806 patients with A1C values available, 51.5% had an A1C < 7.0%. Of patients who were not receiving pharmacologic treatment (n=3256), 64.3% had an A1C < 7.0%. In contrast, A1C was < 7.0% in only 44.0% of patients who received antidiabetes treatment during the study period (n=5550). Of patients being treated with a single medication during the study period (n=3091, 26% received insulin), only 52.2% had an A1C <7.0%; mean distance from A1C target was 1.4 percentage points in those with A1C ≥ 7.0%. Only 33.7% of patients who received two or more antidiabetes treatment classes (n=2459, 36% received insulin) had an A1C <7.0%. Mean distance from A1C target was 1.6 percentage points.
Conclusions: Regardless of intensity of treatment, many patients receiving antidiabetes medication had A1C values above the current ADA recommended A1C target. The observed mean distance from A1C target was substantial in those above target. There is a need for continued aggressive treatment and increasingly effective therapies in order to narrow the treatment gap and better control glucose levels in patients with diabetes.

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Immigrants from the Middle-East have a more severe form of type 2 diabetes than Swedish-born patients
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Background and Aims: Malmo is a multicultural society in the south of Sweden, with different ethnic groups and an overrepresentation of immigrants from the Middle-East. The aim of the study was to compare the clinical characteristics of type 2 diabetes between immigrants from the Middle-East and Swedish-born patients.
Materials and Methods: The study group included 450 consecutive patients with type 2 diabetes [379 Swedish-born aged 61 ± 12 years (143 females 236 males), 71 patients originally from the Middle- East (Lebanon, Iraq, Iran, Syria, Turkey, Egypt), aged 50 ± 11 years (28 females, 43 males)] from the diabetes clinic of Malmo university hospital. In addition to a structured history and physical examination, fasting blood samples were taken for measurement of plasma glucose, HbA1c, serum C-peptide, lipids and creatinine. Albumin excretion rate (AER) was measured from overnight timed urine collection. Fasting values for glucose and C-peptide were used to estimate β-cell function and inulin resistance by homeostasis model assessment(HOMA). All data were adjusted for age, age at onset and BMI.
Results: Onset of diabetes occurred 12 years earlier in the Middle-East immigrants (43 ± 10 Vs 55 ± 12 years, $P<0.001$) compared to the Swedish-born patients. Immigrants had lower fasting serum C-peptide (0.81 ± 0.54 Vs 1.03 ± 0.57 nmol/l, $P=0.013$), lower HOMA-β (2.79 ± 3.32 Vs 4.02 ± 5.42, $P= 0.010$), lower HOMA-IR (0.39 ± 0.26 Vs 0.50 ± 0.34, $P=0.005$), lower systolic blood pressure (135 ± 24 Vs 145 ± 21 mmHg, $P<0.001$) and lower diastolic blood pressure (77 ± 13 Vs 81 ± 10 mmHg, $P=0.003$) than the Swedish group. A first degree family history for diabetes was reported in 61% of immigrants, compared with 47% of Swedish-born($P=0.022$). No significant

differences between the groups were observed in BMI, HbA1c, lipids or AER.
Conclusion: Immigrants from the Middle-East have an earlier onset, stronger family history and more rapid decline of β -cell function than Swedish-born patients suggesting that they have a more severe form of type 2 diabetes than the Scandinavians.

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Great variation in the prevalence rates of diabetes and of glycaemic control in ethnic minority groups in Norway
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Background and Aims: The prevalence of diabetes has been reported to be high among immigrants from several Asian countries to Europe. For the first time we report the age-standardized prevalence of known and newly-diagnosed diabetes in the five largest immigrant groups in Oslo, Norway (Norwegian population 2000 as standard population). Furthermore, we have assessed glycaemic control in subjects with known diabetes in Norwegian subjects living in Oslo and in the five largest immigrant groups.
Materials and Methods: Two population-based, cross-sectional studies were performed in 2000–2002. In the Romsås in Motion study 6140 subjects between 31 and 67 years living in two districts of Oslo were invited and 2950 attended, of whom 78% were of Western (mostly Norwegian) origin. In the Oslo Immigrant Health study a total of 7890 persons born between 1942 and 1971 in Turkey, Iran, Pakistan, Sri Lanka and Vietnam and living in Oslo where invited to a health survey, of which 3019 attended. Diabetes prevalence and glycaemic control were assessed from questionnaires and measurements of glucose and HbA1c.
Results: The age-standardized prevalence of diabetes for 30–60 year olds was lowest in subjects from Norway and Iran, and highest in subjects from Sri Lanka and Pakistan (table 1). The total diabetes prevalence varied tenfold between 2.2% in women from Iran and 21.5% in women from Pakistan. The ethnic differences persisted also after adjustment for waist/hip ratio. Norwegian men had the highest prevalence of undiagnosed diabetes (47.5% of the total diabetes prevalence in the group). Among those with known diabetes, glycamic control was poorest among subjects from Pakistan, who had a mean (SD) level of HbA1c of 8.5 (1.6)%, while the glycaemic control was best among subjects from Iran and Vietnam who had a mean HbA1c of 7.0 (1.6)%.
Conclusion: We found a tenfold variation in the prevalence of diabetes between different immigrant groups living in Oslo, with the highest prevalence rates in women from Pakistan. The level of glycaemic control differed significantly between the groups, with poorest control in subjects from Pakistan, in whom 81% of subjects had HbA1c>7.0%. Our findings highlight the tremendous challenge for diabetes prevention and improvement in diabetes treatment in some immigrant groups.

Table 1. Age-standardized prevalence of diabetes for 30–60 year olds in different ethnic groups

Country of birth	Known diabetes Men		Women		Known + survey-diagnosed diabetes Men		Women	
	Prev	95%CI	Prev	95%CI	Prev	95%CI	Prev	95%CI
Norway	3.1	1.9–4.3	2.0	1.2–2.8	5.9	4.2–7.5	2.9	1.9–3.9
Iran	3.0	1.2–4.7	2.2	0.3–4.0	3.9	1.9–5.9	2.2	0.3–4.0
Vietnam	6.1	3.1–9.1	5.0	2.5–7.6	8.7	5.2–12.3	6.0	3.3–8.8
Turkey	8.3	4.7–11.9	9.9	5.7–14.1	10.4	6.5–14.4	10.8	6.4–15.2
Pakistan	9.8	6.1–13.5	13.6	8.7–18.4	15.3	10.7–19.8	21.5	15.7–27.3
Sri Lanka	13.2	10.5–16.0	13.3	9.9–16.7	17.6	14.6–20.6	15.2	11.6–18.8

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Abstract withdrawn

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Trends of therapy, complications and mortality in elderly type 2 diabetic patients diagnosed in the 80's and in the 90's
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Background and Aims: The therapy of Type 2 Diabetes Mellitus has improved in last years. New treatments and glycemic goals have created an opportunity to better manage this prevalent, chronic disease. There are used wider variety of oral antidiabetics and insulin therapy has been used more frequently, even in elderly patients. The result is a better glycemic control, less complications and deaths. The aim of this study was to investigate the trends in therapy, complications and mortality in 2 cohorts of elderly type 2 diabetic patients aged 60 years or more at time of diagnosis.
Materials and Methods: Records of 2 cohorts of type 2 diabetic patients aged 60 years or more at time of diagnosis were screened for therapy (diet, biguanide, insulin secretagogues, thiazolidinediones, oral therapy association, insulin, insulin associated with oral therapy), complications (retinopathy, nephropathy, chronic kidney failure, neuropathy, amputation, ischemic heart disease, stroke) and death causes (cardiac causes, stroke, infections or septicaemia, cancer, hyperglycaemic coma, other causes). Cohort A included patients diagnosed between January 1st, 1980 and December 31st, 1989 and cohort B, patients diagnosed between January 1st, 1990 and December 31st, 1999. We compared the two cohorts therapy, complications and death causes using statistical analysis.
Results: Mean of 10 certain glycemic values was significantly decreased in cohort B vs. cohort A (p=0.021). In cohort B was used more frequently new oral medication and less number of patients was treated with diet vs. cohort A. Ischemic heart disease was very significant increased in cohort B (p=0.0058). Other complications varied insignificantly between the two cohorts. The rate of death was extremely significant lower in cohort B. Cardiac causes of death and stroke was significantly lower in the second period (p=0.0149, respectively 0.0143). The other death causes varied insignificantly between the two cohorts.
Conclusion: Our results suggest that therapy was improved in elderly type 2 diabetic patients, a better glycemic control was acquired and a significant decrease in rate of death was obtained. Type 2 Diabetes Mellitus in elderly is still associated with a lot of complications.

Table 1. Therapy

n	Cohort A 652	Cohort B 890	Cohort A vs. Cohort B p (Fisher's test or chi-square test)
Mean glycemic value mg%	177.94+/-89	169.22+/-41.25	0.021 (S)
Diet n (%)	200 (30.67)	197 (22.13)	0.0045 (VS)
Biguanide n (%)	424 (65.03)	501 (56.29)	0.0926 (NS)
Insulin secretagogues n (%)	198 (30.07)	428 (48.09)	< 0.0001 (ES)
Thiazolidinediones n (%)	0 (0)	34 (3.82)	< 0.0001 (ES)
Oral therapy association n (%)	115 (17.64)	227 (25.51)	0.0035 (VS)
Insulin n (%)	32 (4.9)	78 (8.76)	0.0067 (VS)
Insulin associated with oral therapy n (%)	3 (0.46)	2 (0.22)	0.6558 (NS)

Tabel 2. Complications and mortality

n	Cohort A 652	Cohort B 890	Cohort A vs. Cohort B p (Fisher's test or chi-square test)
Retinopathy n (%)	72 (11.04)	96 (10.78)	0.9345 (NS)
Nephropathy n (%)	16 (2.45)	15 (1.68)	0.3594 (NS)
Chronic kidney failure n (%)	10 (1.53)	7 (0.78)	0.2179 (NS)
Neuropathy n (%)	69 (10.58)	107 (12.02)	0.4684 (NS)
Amputation n (%)	6 (0.92)	19 (2.13)	0.0694 (NS)
Ischemic heart disease n (%)	150 (23)	314 (35.28)	0.0002 (ES)
Stroke n (%)	33 (5.06)	35 (4.26)	0.3183 (NS)
Death of all causes n (%)	204 (31.28)	57 (6.4)	< 0.0001 (ES)

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Incidence of cardiovascular disease in type 2 diabetes in relation to metabolic parameters and the presence of metabolic syndrome: a 5 year follow up study

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Background and Aims: Cardiovascular disease (CVD) is the main cause of morbidity and mortality in patients with diabetes and metabolic syndrome. The aim of the study is to investigate the incidence of CVD and metabolic syndrome (MS) in patients with type 2 diabetes and its relation to glycemic control and other risk factors.
Materials and Methods: We followed 339 patients with type 2 diabetes without CVD at baseline for 5 years. Mean age: 55.4 ± 14.4 years, mean duration of diabetes: 6.9 ± 7.6 years. CVD was defined as myocardial infarction, stroke, PTCA, by-pass surgery or abnormal resting ECG judged by the Minnesota code criteria for ischemia. MS was defined by modified NCEP criteria (eg: fasting glucose, blood pressure, HDL cholesterol, triglyceride and BMI). Fasting glucose, HbA1c and blood pressure were measured 3–4 times a year and lipid profile was evaluated once a year.
Results: During the follow up period 63 patients had experienced CVD events (CVD+ patients): 18.6%. The CVD+ patients were compared with the CVD- patients at baseline and at the end of the study, after adjustment for age and duration of diabetes. At baseline glucose control (fasting glucose: 187.5 vs 187.0 mg/dl and HbA1c: 8.3 vs 8.2% $p>0.05$) and lipid profile (total, LDL, HDL and nonHDL cholesterol and triglycerides) were not different between the two groups. Systolic blood pressure was higher: 137.3 vs 132.8 mmHg. $p<0.05$ in patients with CVD, while diastolic blood pressure was not different between the groups. During the follow up period CVD- patients had better diabetes control: mean HbA1c: 6.8 vs 7.2% $p=0.05$ and better HDL cholesterol: 49.5 vs 42.2 mg/dl. $p<0.05$, while no difference was found in blood pressure and in other lipid parameters. The metabolic syndrome was present at baseline in173 patients (51.0%) while at the end of the study in 167 patients (49.3%). However during the follow up period 34 patients from those who did not have MS at baseline developed MS, while 40 from those who had MS at baseline reversed to non-MS. The incidence of CVD in patients with MS at year 5 was higher than in patients without MS at year 5 (24.6% vs 12.8% $p<0.01$). In those with MS at baseline the risk of developing CVD was 27% as compared with patients without MS at baseline 9% (OR: 3.8 $p<0.01$). Furthermore patients who remained without MS throughout the study and patients who had MS at baseline but reversed to non-MS, compared to patients who had MS throughout the study and patients who acquired MS during the study had significantly less incidence of CVD.
Conclusion: In patients with type 2 diabetes: 1) the incidence of cardiovascular disease over a 5 year period is associated a) with worse glycemic control b) worse HDL cholesterol and c) the presence of metabolic syndrome. 2) having or acquiring MS increases the risk of CVD, while not having or reversing MS protects from CVD

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Pulse pressure and mortality in hypertensive type 2 diabetic patients. A cohort study

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Background and Aims: Hypertension is a well-known cardiovascular risk factor in type 2 diabetic patients. It has been suggested that pulse pressure (PP) could be an independent cardiovascular risk factor in the general population, particularly in the elderly. An association between office PP and cardiovascular mortality has been previously reported in diabetic patients, while the relationship between ambulatory measurements of PP and all-cause mortality has not been assessed so far.
Materials and Methods: A cohort study was performed on a consecutive series of 435 diabetic outpatients. All patients underwent office blood pressure measurement (OBP) and 24-h ambulatory blood pressure monitoring (ABPM). Mortality was assessed through queries at the Registry Offices of the city of residence for each patient. Mean follow-up was 3.8 ± 1.2 years.
Results: Fifty-eight patients (13.3%) deceased during follow-up. Mortality was significantly ($p<0.05$) higher in patients in the highest quartile, and lower in the lowest quartile, when compared to intermediate quartiles, both for office and ABPM-PP. In a multivariate analysis, after adjustment for numerous variables (including current hypoglycaemic, antihypertensive statin and aspirin treatment), mortality was increased of 3.1% and 5.3% for each incremental mm Hg of office PP ($p<0.05$) and ABPM-PP ($p<0.001$), respectively.
Conclusion: High PP, assessed through office measurement or ABPM, was associated with increased mortality in hypertensive type 2 diabetic patients. In our sample, PP assessed with ABPM is a better predictor of mortality than office PP.

PS 60
Nutrition and diet I

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Glucose-induced cannabinoid receptor 1 repression in peripheral blood leukocytes

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Background and Aims: The Cannabinoid receptor 1 (CB1), which is predominantly expressed in the brain, in adipose tissue and immune cells, is involved in the regulation of immunity, appetite and body weight. Data from animal studies demonstrate that CB1 expression is upregulated in adipose tissue of obese rats and that CB1 knockout mice eat less than their wildtype littermates. A higher food intake was shown for young mice, compared to older or CB1 knockout mice. Application of the CB1 antagonist SR141716A (Rimonabant) induces reduction of food intake and weight loss, which is associated with a decrease of serum low density lipoprotein, insulin and glucose levels. So far it is unclear if CB1 expression is directly regulated by nutritional stimuli and the aim of this study was (1) to test the hypothesis that CB1 expression in peripheral blood cells can be modulated by glucose uptake, and (2) to investigate whether the response to glucose is associated with sex, age, BMI or metabolic parameters.
Materials and Methods: The study population (n=77; 46 male/31 female; age 45.6 ± 8.9 years, BMI 28.3 ± 4.5 kg/m^2) with normal glucose tolerance received a 75g oral glucose tolerance test (OGTT). CB1 mRNA expression in peripheral blood leukocytes was measured by quantitative RT-PCR before (0 h) and 2 h after glucose load and the CB1 expression ratio (2 h / 0 h) was determined.
Results: We found a significant decrease of CB1 expression ratio on response to glucose in young subjects only (age ≤ study median of 47 years), and rather an increase in older subjects (mean CB1 expression ratio 0.88 ± 0.32 in young *versus* 1.24 ± 0.71 in older subjects; p=0.0057) which was not associated with sex, BMI, waist circumference, systolic or diastolic blood pressure or concentrations of triglycerides, uric acid, 0 h or 2 h blood glucose.
Conclusion: It can be concluded that CB1 expression is downregulated by oral glucose challenge in young subjects but this regulation seems to be impaired or even inverted in older subjects. There is a need for further studies to clarify the mechanism of age-dependent glucose-regulation of CB1 expression and possible nutritional and immunological consequences concerning type 2 diabetes mellitus and atherosclerosis.

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Differential regulation of leptin levels by carbohydrate foods: control trials in diabetic and non diabetic men

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Background and Aims: It has been suggested that low-glycaemic index (LGI) diets could reduce satiety. Moreover, we demonstrated previously that a long-term LGI diet decreased total fat mass and tended to increase lean body mass in healthy overweight, but not in diabetic subjects (T2D). Leptin modification could be implicated in these results. Therefore, we questioned whether the profile of plasma leptin might differ in response to high (H) or LGI diets, in both normal and T2D subjects. This study is part of two randomized controlled trials.
Materials and Methods: Two groups of subjects volunteered to participate in the present study: a group of 11 healthy overweight subjects (BMI 28 ± 1 kg · m^{-2}, fasting glycaemia 5.6 ± 0.1 mmol, mean±SEM), and a group of 12 T2D men (BMI 31 ± 1 kg · m^{-2}, fasting glycaemia 8.7 ± 0.7 mmol). Subjects in each group were randomly allocated to two periods of 4–5 weeks of a LGI- or a HGI diet in a crossover design. The two periods were separated by a washout interval of 4–5 weeks.
Results: In the healthy subject, during the first day profile, postprandial plasma leptin was lower with the LGI than with the HGI meals (acute effect). Consistently, the incremental AUC for plasma leptin after the LGI diets was lower (p<0.0001) than that with the HGI-meals. At the end of the nutritional period, low-GI diets lowered significantly plasma leptin profile and the AUC was decreased (1st day vs 5 wk data, p<0.05; delta-LGI vs delta-HGI, p<0.05). During the HGI dietary period, there were no modifications in plasma leptin levels. In T2D subjects, no modification in leptin levels could be detected during either the LGI or HGI diets, neither between the two diets at any time of the study.
In conclusion, in slightly overweight subjects, LGI diets induced low plasma leptin levels compared to a HGI diet after one-day profile as well as after longer-term period. In diabetic subjects these diets could not modify plasma leptin levels. Thus, contrary to what has been sought, low leptin levels during LGI diets could not be implicated in reducing satiety, but might be the consequence of reducing total fat mass.
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The effect of 'pre-dinner drinks' on postprandial glycaemia and insulinaemia

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Background and Aims: Moderate consumption of alcoholic beverages has been associated with reduced the risk of cardiovascular disease. This beneficial effect has been partly explained by a direct effect of alcohol on lipidemia and thrombogenesis. Just as important could be an effect on hyperglycaemia and insulin resistance, which are also recognised risk factors for heart disease. Previous studies have focussed on alcohol consumption with a meal, rather than before a meal (the "pre-dinner drink"). The aim of the present study was to determine how the consumption of 2 standard drinks of alcohol (20g) 1 hour before a meal would affect the glycaemic and insulinaemic response to that meal. We also aimed to determine if there were any differences in this effect for 3 common beverages: beer, wine and gin.
Materials and Methods: Eight healthy subjects (age 18–25 years and BMI kg/m^2; 18–25) participated in the study. Each subject undertook 5 treatments (beer, wine, gin and 2 control trials with water) on separate occasions in random order. On each test day, a standard pre-test breakfast was given, followed by the test beverage 3 hours later. One hour after the test beverage, a standard meal based on a 75g carbohydrate portion of mashed potato was consumed, and the glucose and insulin response measured at regular intervals over 2 hours.
Results: Following a pre-meal drink of water (the control trial), the area under the response curve (AUC) for plasma glucose was 244 ± 14 mM.120 min (mean±SEM). In the beer trial, the AUC was reduced by 25% (182 ± 19 mM·120 min, p=0.01), in the wine trial by 23% (189 ± 19 mM.120 min, p=0.03), and in the gin trial by 19% (198 ± 19 mM·120 min, p=0.06). Significant changes were also seen in peak plasma glucose, and the 2-hour glucose concentration. Despite the marked reduction in glycaemia, insulinaemia was not decreased. Indeed in the gin trial, insulinaemia was increased by 65% compared with the control trial (213 ± 26 vs. 129 ± 18, p<0.05, respectively).
Conclusion: This study suggests that the consumption of an alcoholic beverage 1 hour before a meal significantly reduces the glycaemia but not insulinaemia. The reduction is greater for beer and wine than for gin. If confirmed, our findings suggest an alternative mechanism by which alcoholic beverages might contribute to a reduced risk of cardiovascular disease.

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The fatty acid composition of plasma is related to obesity and metabolic disorders in adolescent boys and girls

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Background and Aims: The amount as well as the quality of dietary fat has been related to development of obesity and metabolic disorders, but dietary surveys are often imprecise. Another method for estimating dietary fat is to measure the fatty acid composition in plasma. In this study the relationships of plasma fatty acids with obesity, and metabolic variables related to the metabolic syndrome, were assessed in 13–17 year old boys and girls in an effort to clarify factors related to the early development of these disorders.
Materials and Methods: The subjects were randomly selected Minneapolis, MN students (N=285) participating in a study of the metabolic syndrome. They underwent anthropometric measures and an euglycemic insulin clamp at which time blood was obtained for fasting insulin, glucose, lipids

and fatty acids. The fatty acid composition of the plasma cholesterol esters was analysed by gas-liquid chromatography. Relationships between the fatty acid composition and metabolic variables were studied using Spearman correlations and linear regression analysis.
Results: In boys, but not in girls, there were strong significant relationships between the fatty acid composition and obesity (BMI, waist circumference, % body fat) while both sexes showed significant relationships to triglycerides and fasting insulin, which remained after adjustment for BMI and physical activity. There were virtually no relationships to plasma glucose, insulin sensitivity (adjusted for lean body mass) or BP blood. In general, a fatty acid profile characterized by an increased proportion of saturated fatty acids, low levels of linoleic acid (18:2 n-6) and with high proportions of palmitoleic (16:1 n-7) and gammalinolenic (18:3 n-6) acids indicating increased activities of delta-9 and delta-6 desaturase, respectively, was related to obesity (in boys) and increased triglycerides and insulin levels in both boys and girls. The relationships between the proportions of 16:1 n-7 and 18:3 n-6, respectively, and triglycerides after adjustment for BMI were for boys r = 0.34, p<0.0001 and r = 0,32, p<0.0001 and for girls r = 0.36, p<0.0001 and r=0.28, p=0.002.
Conclusion: The data show that the fatty acid pattern, which partly reflects dietary fat quality, already in adolescence is associated with increased cardiovascular risk, also independent of BMI. Strong relationships between fatty acid composition and anthropometric variables were present in boys, but not in girls. While there were significant relationships between the fatty acid pattern and triglyceride and isulin concentrations in both sexes, similar relationships to insulin sensitivity and blood pressure were lacking. The relationships between fatty acid composition in plasma and metabolic variables were qualitative similar to those observed in adult populations. This supports the suggestion that dietary intervention early in life may be needed to reduce the increasing development of obesity and the metabolic syndrome.

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Improvement in glucose tolerance, serum free fatty acid levels and body fat composition with a novel palatinose-based balanced formula

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Background and Aims: Many studies have shown that sustained hyperglycemia is a risk factor for both microvascular and macrovascular (or cardiovascular) complications in type 2 diabetes (DM), while postprandial hyperglycemia has also been considered a risk factor for cardiovascular complications. Many experimental and epidemiological studies have shown that increased postprandial plasma glucose levels may have equally or even more harmful effects than fasting hyperglycemia, and the reduction of postprandial plasma glucose levels delays the development of cardiovascular complications. Postprandial hyperglycemia is a recognized consequence of the effects of both insulin resistance and the impairment of early insulin secretion in response to an oral glucose load. However, inversely, both insulin resistance and the impairment of early insulin secretion in response to an oral glucose load can, at least initially, be improved by reducing postprandial glucose levels. Palatinose (isomaltulose) is an oligosaccharide present in honey, which has shown promise as a noncariogenic caloric sweetener. Although palatinose is completely absorbed, it has the specific characteristics of delaying digestion and absorption. We have developed a novel palatinose-based balanced formula (PBF) for a possible therapeutic means to improve postprandial hyperglycemia, and its accompanying conditions and complications. We conducted a crossover study to examine whether PBF has beneficial effects on glucose tolerance, lipid profile and body fat composition in humans.
Materials and Methods: Twenty-three subjects with impaired glucose tolerance (IGT) were randomized to consume PBF (200 kcal) at breakfast while maintaining their usual total energy intake (Intervention) or their usual breakfast (Control) for 12 weeks before crossover. Changes in the trait values were evaluated to measure the effects between different subjects in a parallel across-arm comparison of Intervention vs. Control, as well as serially in the same subjects in a crossover comparison of the periods.
Results: The intervention significantly decreased 2-hr plasma glucose levels after OGTT (Intervention vs. Control: -15.7 ± 20.1 vs. 0.8 ± 31.6%, p=0.038, and -15.7 ± 20.1 vs. 13.4 ± 30.2%, p=0.021, for analyses between different subjects and the same subjects, respectively) and serum free fatty acids (FFA) levels (-22.3 ± 21.5 vs. 18.7 ± 52.1%, p=0.017 and -22.3 ± 21.5 vs. 26.4 ± 43.6%, p=0.010). The decrease in the abdominal visceral fat area (VFA) with the intervention was not significant. However, in subjects whose VFA decreased in the intervention period and increased after the intervention (responder), VFA (101.6 ± 27.0 vs. 53.7 ± 33.9 cm^2, p=0.016) at the baseline was significantly higher than in the other subjects.
Conclusion: PBF consumption is a promising therapeutic means of decreasing postprandial hyperglycemia and serum FFA levels and, at least in viscerally obese subjects, VFA.

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Effects of saturated and monounsaturated fat rich diets on insulin sensitivity and meal lipid tolerance in type 2 diabetic patients

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Background and Aims: Monounsaturated fat (MUFA) rich diets, compared with saturated fat (SAFA) rich diets, improve insulin sensitivity in healthy individuals. Less is known on this aspect in type 2 diabetic patients. Moreover, the effects of these two diets on postprandial lipid metabolism is still matter of debate. Therefore, the aim of this study was to evaluate the effects of MUFA and SAFA rich diets on insulin-resistance and postprandial lipemia in type 2 diabetic patients.
Materials and Methods: Ten type 2 diabetic patients (age range 41-65 yrs, in stable metabolic control with diet and/or oral hypoglycaemic drugs) were given, in a random order, two diets, one rich in MUFA (SAFA 8%, polyunsaturated fatty acids 4%, MUFA 23%) and the other rich in SAFA (SAFA17%, polyunsaturated fatty acids 3%, MUFA 15%). Each diet was followed for 3 weeks. At the end of the two diets, a euglycemic hyperinsulinemic clamp was performed and, on another day, a standard fat rich meal (944 kcal, 57% fat, 31% CHO, 12% protein) was administered to the patients. Before and for six hours after the meal, blood samples were taken for determination of cholesterol and triglycerides in plasma and in different lipoproteins, separated by discontinuous density gradient ultracentrifugation.
Results: Blood glucose control and body weight did not change during the study period. Insulin-resistance was similar at the end of the SAFA and MUFA diets (M value: 4.7 ± 0.6 and 4.0 ± 0.4 mg/kg b.w./min) (M+SEM). The two diets did not induce any significant change in the meal lipid tolerance (similar chylomicron and large VLDL response), but for the cholesterol and triglyceride incremental areas of small VLDL, which were significantly lower after the MUFA diet compared with SAFA (cholesterol: -8.72 ± 2.34 vs. -4.70 ± 2.02 mg/dl•6 hrs, p<0.05; triglycerides: -13.58 ± 4.70 vs. -2.22 ± 3.75 mg/dl•6 hrs, p<0.005).
Conclusion: A MUFA rich diet does not seem to improve insulin-sensitivity in type 2 diabetic patients, who are characterized by a well established insulin resistance. For what concerns postprandial lipid tolerance, this diet significantly reduces the postprandial response of small VLDL lipoproteins, which are considered more atherogenic.

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A low carbohydrate compared with a low fat diet in elderly patients with type 2 diabetes mellitus

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Background and Aims: Type 2 diabetes mellitus in older people is a growing medical problem. This study was designed to evaluate the effects of low carbohydrate or low fat diet on weight loss and risk factors for atherosclerosis in elderly, obese patients with type 2 diabetes mellitus.
Materials and Methods: A number of 64 patients, 24 men (37.5%) and 40 women (62.5%) with an average age 65.5 ± 4.2 years, obese (BMI>30 kg/m^2) with hypertriglyceridemia (TG>160 mg/dl), type 2 diabetes mellitus (glycemia>110 mg/dl) and hypertension (systolic BP>140 mmHg and diastolic BP>90 mmHg) were included into an educational program consisting of carbohydrate restricted diet or a calorie and fat restricted diet. Every patient included in this program was clinically reevaluated every 2 months. Measurements of blood pressure, glycemic and lipid levels, were taken after 6 months.
Results: Patients on the low carbohydrate diet lost more weight during the six month study (6.83 ± 1.61 kg vs. 5.16 ± 0.92 kg, p<0.05) than did those on the low fat diet. During the six month study, there was a greater decrease in the mean triglyceride levels in the low carbohydrate group (229 ± 29 mg/dl to 163 ± 17 mg/dl, p<0.01) than in the low fat group (206 ± 23 mg/dl to 186 ± 26 mg/dl, p<0.01). The mean fasting glucose level decreased more in the low carbohydrate group (149 ± 25 mg/dl to 129 ± 19 mg/dl, p<0.05). We

did not observe significant overall or between group changes in blood pressure.
Conclusion: Severely obese type 2 diabetes elderly patients lost more weight during six month on a carbohydrate restricted diet than caloric and fat restricted diet, with a relative improvement in insulin sensitivity and triglyceride levels.

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Impact of short term fat absorption on neural and endocrine regulators of energy homeostasis
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Background and Aims: Insulin, leptin, serotonin (5-HT) and melanocortins in close interaction, negatively affect energy homeostasis, acting mainly on the hypothalamus. Genetic and environmental factors, including nutrients, control the peptide-neurotransmitter interactions. Disturbances of these homeostatic mechanisms result in the development of metabolic dysfunctions and ultimately in severe pathologies. The proportion of fat intake and the ratio of fat versus the other macronutrients in a diet, are of importance in these dysfunctions. The present studies focus on the impact of fat absorption for a short period, on the mechanisms operating in the above pathophysiological processes.
Materials and Methods: *In vivo* experiments. Adult male Wistar rats, were fed a low fat (LF) chow laboratory meal, 5% fat, 59% carbohydrate (CHO). A high fat (HF), 40% fat, 17% CHO, diet was given for 7 days. The HF-fed rats had a lower food intake, but a higher calorie intake than LF fed rats. Body weight was similar in the two groups. Microdialysis studies were performed in awake rats. One week before the microdialysis procedure, a guide was placed stereotaxically under anesthesia, in the median hypothalamus (Paraventricular nucleus, Ventro median hypothalamus). The extracellular hypothalamic response of 5-HT to a meal was determined in the dialysates by HPLC-electrochemical detection. *In vitro* studies: 3 Hypothalami / 0.20 ml type Ringer buffer containing 0.2% BSA, were preincubated during 20 min in presence of 11 mM glucose and subsequently were incubated for 15 min with 22 mM glucose, at 37°C, O2 95%. Insulin was radioimmunoassayed in the incubation medium. In another series of experiments, after decapitation, the hypothalami were dissected and plunged in liquid nitrogen. Following extraction, mRNA was assessed by real-time RT-PCR.
Results: The HF diet increased basal circulating leptin levels, basal hypothalamic 5-HT release and attenuated dramatically the hypothalamic 5-HT response to the chow meal. These alterations are similar to those observed previously following the consumption of a single exclusively animal fat meal (lard). In this latter case the hypothalamic 5-HT response to a chow meal was significantly attenuated 24 h later, and the circulating leptin levels were altered. In addition, in the present study, the HF diet increased the gene expression of insulin and of leptin receptor and the insulin release *in vitro* by the hypothalamus. Basal glycemia and insulinemia were identical in the two groups.
Conclusion: The present work shows that a HF, low CHO diet affects rapidly central and peripheral regulators of energy and glucose homeostasis. These changes might represent either compensatory homeostatic mechanisms or early alterations, that if persist, could result in permanent neural, endocrine or metabolic disorders, in particular in organisms with genetic predisposition.

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Dietary composition and development of type 2 diabetes in high-risk subjects – The Finnish Diabetes Prevention Study (DPS)
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Background and Aims: Several lifestyle factors are known to be associated with type 2 diabetes risk, but data on high-risk subjects who constitute the target of the potential preventive intervention are scarce. The aim of this study was to assess the independent effect of dietary macronutrient composition on type 2 diabetes incidence during an intervention trial aiming at diabetes prevention by lifestyle modification.
Materials and Methods: Altogether 522 overweight, middle-aged men and women with impaired glucose tolerance (IGT) were randomised either to get 'usual care' (control) or intensive lifestyle counselling to reduce weight, to increase physical activity and intake of dietary fibre, and to decrease intake of dietary fat and saturated fat. For this analysis the treatment groups were combined. Dietary intake by 3-day food records was measured at baseline and annually and mean intakes of fibre (g/1000 kcal), total fat (E%), and saturated fat (E%) during the intervention were calculated. Diabetes status was assessed by repeated 75-g oral glucose tolerance test at annual clinic visits. The Cox model was used to analyse the relationship between quartiles of fibre, fat and saturated fat intake and risk of getting diabetes during the mean follow-up of 3.9 years. Furthermore, to estimate the combined effect of fat and fibre intake, individuals were divided by median into four categories: low-fat/high-fibre, low-fat/low-fibre, high-fat/high-fibre, and high-fat/low-fibre. All models were adjuster for sex, treatment group, baseline weight, baseline 2-h plasma glucose, weight change and physical activity.
Results: In separate models, fibre, fat, and saturated fat intake were all associated with diabetes risk. Hazard ratios (highest compared with lowest quartile) were 0.41 (95% CI 0.22–0.79) for fibre intake, 2.47 (95% CI 1.38–4.40) for fat intake, and 1.91 (95% CI 1.06–3.46) for saturated fat intake. Compared with the low-fat/high-fibre category, hazard ratios were 1.95 (95% CI 0.94–4.06), 2.99 (95% CI 1.60–5.56), and 2.18 (95% CI 1.29–3.68) for low-fat/low-fibre, high-fat/high-fibre, and high-fat/low-fibre, respectively.
Conclusion: Dietary fat and fibre intake are significant predictors of progression to type 2 diabetes in high-risk subjects even after adjustment for weight, weight change and physical activity.

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The effect of Greek mediterranean diet on trace elements and blood coagulation factors in type 2 diabetic patients
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Background and aims: DM2 has been associated with altered levels of trace elements and a prothrombotic state.We investigated the effect of a 28-day Greek mediterranean diet (rich in fiber,mono- and polyunsaturated fatty acids and complex carbohydrates) on serum concentration of trace elements and blood factors participating in coagulation and fibrinolysis in 58 patients with DM2.
Materials and methods: Blood trace elements and blood coagulation factors were evaluated in 35 men aged (M±SEM) 61,6 ± 1,57 years and 23 women aged 58,8 ± 1,97 years with DM2,on treatment with diet or/and oral hypoglycaemic agents as well as in 22 healthy controls matched for sex and age.The food,rich in olive oil,vegetable and fruit,was prepared and provided daily by two commercial firms.The diet was isocaloric to the current so that patients could not reduce their weight during the test period.The BMI and HbA1c of patients were less than 28 and 7%.The study subjects were assessed before and after the end of the 28 day-period on the diet.The Wilkoxon matched paired test was applied for the statistical analysis.All values are expressed as means±SEM.
Results: BMI did not change significantly following the diet but waist perimeter was reduced in diabetic men and in the control group.HbA1c fell in the patients (6,45 ± 0,21% versus 7,00 ± 0,18%, p<0,001).Serum ferritin was drastically reduced in the patients (95,24 ± 11,7 versus 109,83 ± 11,08 ng/ml, p=0,0006),whereas plasma levels of Mg and P increased following the diet in patients and controls (p<0,02).Analysis of parameters involved in haemostasis revealed a significant reduction of fibrinogen in patients (374,76 ± 9,78 versus 393,96 ± 10,05,p=0,016) but a decrease in the activity of the anticoagulant proteins C and S both in patients (p<0,001) and controls (p<0,04).
Conclusions: It is concluded that adherence to mediterranean diet even for a short period improves glucose control,induces increases in plasma levels of Mg and P and reduces serum ferritin which is positively correlated to insulin resistance.Additionally it lowers blood fibrinogen levels but also reduces the activity of proteins C and S, an effect the significance of which remains to be elucidated.

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Carnitine deficiency during pregnancy and lactation induces metabolic alteration in neonatal offspring and programmes insulin resistance in adult life

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Background and aims: Carnitine is an endogenous molecule with important functions in normal intermediary metabolism and cellular physiology. Its primary function is to facilitate the transport of fatty acids across the inner mitochondrial membrane, making them available for mitochondrial β-oxidation. Carnitine depletion can be particularly critical in fetal and neonatal life, because placenta and neonatal development are both subjected to intense fat metabolism. Unfavourable nutritional environment in early life is now widely recognised as a risk factor predisposing to abnormal lipid and carbohydrate metabolism, including metabolic syndrome, obesity and type 2 diabetes, in adult life. In order to test the hypothesis that carnitine deficiency in fetal-neonatal life may represent such a risk factor, we administered sodium pivalate (known carnitine depletion inducer) to female rats during pregnancy and lactation.
Materials and Methods: Thirty female Sprague-Dawley rats were divided into 3 groups of 10 animals each, treated during pregnancy and lactation with: 1) 20 mM pivalate in drinking water; 2) 20 mM pivalate plus 40 mM carnitine in drinking water; 3) vehicle ($NaHCO_3$ 20 mM in drinking water). Carnitine levels were determined in placenta, fetus and neonate by HPLC-MS. Insulin levels were determined in serum and pancreas of neonates at 3, 13 and 21 days of age. Morphometric analysis of pancreas was performed on neonates at 21 days of age. After weaning, all rats were maintained on laboratory chow and monitored for serum glucose, insulin and triglyceride levels. At 6 months of age, an Oral Glucose Tolerance Test was performed (OGTT, 2.5 mg/kg b.w.) collecting blood samples from the tail vein at 0, 30, 60, 120 min after glucose load.
Results: Pivalate treatment determines a large reduction of carnitine levels in all dams, placenta, fetus, and in different neonatal tissues (about 60–80% with respect to controls), together with a reduction of placenta, fetus and neonatal weight. There is an increase in triglyceride content in several neonatal tissues. Pancreas from 21 day-old carnitine-depleted neonates shows an increase in Langerhans islets size (10.1 ± 0.6 vs $13.8 \pm 1.3\ \mu m^2 \times 10^3$; $p \leq 0.05$) and total insulin content (20.8 ± 6.7 vs 34.2 ± 12.5 μg/pancreas, $p \leq 0.02$). Carnitine supplementation in pivalate-treated female rats restores carnitine levels in several maternal and neonatal tissues and counteracts abnormalities in lipid metabolism. After weaning, there is progressive hyperinsulinemia (from 1.37 ± 0.64 at 2 months to 3.64 ± 0.69 at 6 months of age) and hypertriglyceridemia (from 85 ± 33 at 2 months to 165 ± 67 at 6 months of age) in rats from pivalate group with respect to controls. Carnitine supplementation counteracts these effects. At six months of age, OGTT shows an insulin resistance state in pivalate group whose development seems to be partially prevented in the group from carnitine-supplemented mothers.
Conclusions: Our data suggest that carnitine depletion during fetal and neonatal life may determine metabolic programming in different pup tissues that could lead to profound alteration in glucose and lipid homeostasis in adult life (insulin resistance status).

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Nutritional status of moderate to severe renal insufficiency patients with diabetic nephropathy

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Background and Aims: Dietary regulations especially protein restriction is generally advocated in patients with chronic renal insufficiency (CRI) to retard renal decline and ameliorate uremic symptoms. In this study we included a group of diabetic nephropathy (DN) patients to evaluate their protein-calorie nutritional status resulting from a pre-prescribed dietary advice.
Materials and Methods: A number of DN patients with relatively stable CRI were recruited These patients were already on dietary guideline for a variable period of time, which advised calorie intake of 20–30 kcal/kg/day, protein 0.4–0.5 g/kg/day, phosphate & salt restriction, and calcium & vitamin supplementations. Finally 110 patients were selected and based on creatinine clearence rate (CCr) sub grouped in two categories CRI-3 (moderate renal insufficiency: 31patients with CCr 30–60 ml/min/1.73 m^2) and CRI-4 (severe renal insufficiency: 79 patients with CCr 10–29 ml/min/1.73 m^2). A 3-day dietary diary, anthropometric measure, clinical evaluation and relevant laboratory parameters were done in these patients.
Results: In the two groups of CRI-3 & CRI-4 patients the age (57 ± 9 vs. 58 ± 8 years), diagnosed duration of diabetes (13 ± 8 vs. 12 ± 8 yrs), hypertension (7 ± 4 vs. 9 ± 7 yrs) and renal failure period (3 ± 2 vs. 3 ± 2 yrs) were similar (P=NS). Other similar parameters were HbA1c (6.6 ± 1.5 vs. $7.6 \pm 2.2\%$), C-reactive Protein (10 ± 8 vs. 12 ± 11 mg/l), Transferrin (193 ± 65 vs. 188 ± 67 μg/dl), Albumin (3.6 ± 0.5 vs. 3.5 ± 0.5 g/dl), Calcium (8.8 ± 1 vs. 8.7 ± 1 mg/dl), Phosphate (4.1 ± 1 vs. 4.2 ± 1 mg/dl), Triglyceride (215 ± 128 vs. 187 ± 102 mg/dl) and Cholesterol (181 ± 59 vs. 179 ± 46 mg/dl) (P=NS). Parameters those differed between CRI-3 and CRI-4 groups were BMI (26 ± 4 vs.24 ± 3 kg/m^2 P<0.01), Serum Creatinine (2.1 ± 0.5 vs. 3.9 ± 1.1 mg/dl, P<0.001), Hemoglobin (11.1 ± 1.7 vs. 9.9 ± 1.8 g/dl, P<0.007), Serum Total Protein (7.1 ± 0.5 vs. 6.7 ± 0.7g/dl, P<0.01) and Urinary Protein excretion (1.4 ± 1.1 vs. 2.6 ± 2.1 g/day, P<0.001). Anthropometric comparison included mid arm circumference (28 ± 2.3 vs. 26 ± 2.7 cm, P<0.02), biceps (12 ± 3 vs. 9 ± 4 mm, P<0.02) triceps (18 ± 6 vs. 15 ± 6 mm, P<0.05) & sub scapular (30 ± 6 vs. 33 ± 8 mm, P<0.02) skin fold thickness and waist-hip ratio (0.96 ± 0.1 vs. 0.90 ± 0.1, P<0.03) which was higher in moderate insufficiency group. Dietary diary of CRI-3 and CRI-4 showed similar calorie (21 ± 4 vs.21 ± 7 kcal/kg/day), total protein (0.8 ± 0.2 vs.0.8 ± 0.4 g/kg/day), high biological value protein (0.31 ± 0.1 vs.0.37 ± 0.2 g/kg/day), carbohydrate (3.6 ± 0.8 vs.3.5 ± 1.4 g/kg/day) and fat intake (0.28 ± 0.11 vs.0.30 ± 0.14 g/kg/day) (P=NS). Correlation studies showed increasing serum creatinine associated with lowering triceps and sub scapular thickness. High creatinine clearance rate positively correlated with greater mid arm circumference, triceps & sub scapular skin fold thickness. Increased urinary protein excretion negatively correlated with serum protein level.
Conclusion: It can be concluded that diabetic nephropathy subjects of severe renal insuffiency are more nutritionally compromised than moderate renal failure patients even with similar dietary pattern. The low protein diet is unlikely to have any effect on renal functional deterioration rather this may predispose this group of patients to malnourishment.
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Patients given nutrition advice in an office setting but with home exercise advice lose more weight than those with both home nutrition and exercise advice

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Background and Aims: In a general practise setting where all weight management involved nutrition advise by a die titian, exercise advice either by a exercise physiologist or physiotherapist and support and motivation by the family physician all in an office setting, this study wished to test the hypothesis that nutrition advice and exercise services taken to the home would result in better weight loss outcomes than the same nutrition and exercise advice given in the office setting.
Materials and Methods: 62 obese patients were offered participation in an enhanced primary care approach to weight management. Patients were randomly selected for either office diet and home exercise advice, office diet and exercise advice or home diet and exercise advice. The Harris Benedict Equation was used. All were referred for 2 dietitian consultations (60 minutes in office or 120 minutes at home) for a meal plan with a 300 kcal deficit, 25 to 30g fat for females and 35 to 40g fat for males per day, mono-unsaturated uncooked. Remaining calories were 55% carbohydrate (low GI) and 45% protein. 75% of total energy intake was to be consumed before evening. There was to be no carbohydrate at night unless exercise in excess of the 300 kcals per day prescription had occurred. The exercise physiologist designed an endurance training programme to achieve the 300 kcal/day expenditure. 3 EP consultations totalled 135 minutes. Consultations with AHP's and GP were approximately 10 days apart to optimise motivation and support
Results: Group 1 – Office diet and home exercise comprised 28 (16F and 12M) patients. Median age 62(F) and 61(M). Median start weight 84.7 kg(F) and 84 kg (M). 50% of all female participants lost between 3.2 and 6.7 kg (0.5 and 4.6 kg males) over a 3 month period with median loss of 5.6 kg /6.6% (F) and 3.78/4.5%(M). Group 2 – Home diet and execsie comprised

25 (17F and 8M) patients. Median age 53(F) and 47(M). Median start weight 77 kg(F) and 104 kg(M). 50% off all female participants lost between 1.85 and 4.7 kg (1.3 and 6.5 kg males) over 3 months with median loss 3.0 kg/3.9% (F) and 2.2 kg/2.1% (M). Group 3 – office diet and exercise was a small group comprising 9 females. Median age 66. 50% of these participants lost between 2.0 and 5.4 kg. Medain loss was 2.6 kg/3.3% over 3 months. Weight loss in grams per minute of AHP contact time was greatest for group 1- 28.17 g/min (F), 19.38g/min (M). Group 2 – 11.7g/min (F) 8.62g/min (M) and Group 3- 13.33g/min (F).
Conclusion: The hypothesis was not validated by the outcomes. Patients given the diet advice in an office setting appeared to lose more weight in a 3 month period. Of significance was that the median weight lost per minute of AHP consultation contact time was greatest for the office diet home exercise group (28.17 g/min (F), 19.38g/min (M)) suggesting that the diet advice in an office setting is more successful and cost effective than the home setting (11.7g/min (F) 8.62g/min (M)) whilst exercise advice in a home setting is better received than the same advice given in an office setting.

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The influence of chromium chloride containing milk to glycemic control of the patients with type 2 diabetes: a randomized, double-blind, placebo-controlled trial
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Background and Aims: To evaluate the effect and safety of chromium containing milk powder in patients with type 2 diabetes.
Materials and Methods: A randomized, double-blind, placebo-controlled trial was conducted in Taiwan. Totally, 40 type 2 DM patients, aged 30–75 years, under gliclazide sulfonylurea agent 160 mg/day or less at least 3 months were enrolled. Their HBA1c ranged 7.5–12%, fasting plasma glucose (FPG) 7.78–13.88 mmol/l and BMI 20–35 kg/m^2. The subjects were divided into 2 groups to receive either chromium containing milk powder (chromium 200 μg/20 gm milk powder) or placebo twice a day for 16 weeks. FSIGT (IVGTT) was performed before and after treatment.
Results: The chromium-group demonstrated a lower FPG and FPI (–2.1 ± 0. 2 vs. 3. 5 ± 0. 5 mmol/l, and –10 ± 7.2 vs. 11.1 ± 5.1 pmol/l, $p < 0.05$ respectively), especially in male patients (–2.6 ± 0.2 vs. 4.8 ± 0.7 mmol/l, and –16.1 ± 5.2 vs. 18. 2 ± 4. 2 pmol/l, $p < 0.01$ respectively) at the end of the study. Lower HbA1c was observed in chromium-treated male patients (–1.1 ± 0.5 vs. 0.7 ± 0.2, $p < 0.05$). But there were no significant changes in other metabolic parameters (lipid profiles including total cholesterol, triglyceride, LDL-C, HDL-C) except improvement of insulin resistance (HOMA-IR & S_I from FSIGT) were observed in male patients (–2.1 ± 1.1 vs. 0.41 ± 1.12 & 0.18 ± 0.11 vs. –0.15 ± 0.2, $p < 0.05$, respectively). There were no adverse events in both groups, except for several mild complaints in chromium-group on constipation (5%),flatulence (5%).
Conclusion: Intake of milk powder containing 400 μg/day of chromium for 16 weeks in type 2 diabetic subjects resulted in lowering of FPG, FPI and improvement of metabolic control in male patients.

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Effect of chromium picolinate and biotin combination as an adjunctive nutritional therapy in poorly-controlled patients with type 2 diabetes mellitus
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Background and Aims: Recent clinical trials suggest that chromium picolinate (CrPic) alone and in combination with biotin can enhance insulin sensitivity and reduce elevated blood glucose and cholesterol levels. The blood glucose lowering effect appears to be greatest in poorly controlled patients (≥10% HbA1c). This analysis was conducted to evaluate the effects of the chromium picolinate and biotin combination as an adjuvant nutritional therapy in patients with ≥10% HbA1c.
Materials and Methods: This was a 90-day, randomized, double-blinded, placebo-controlled multi-center prospective study. Study subjects were randomly assigned in a 2:1 ratio to active (600 mcg Cr as CrPic and biotin 2 mg/per day) or placebo groups. Study subjects maintained stable doses of oral antidiabetic medication(s), including: sulfonylureas (SU), biguanides (B), and thiazolidinediones (TZD). Fasting HbA1c, fasting plasma glucose (FPG) and safety information were collected at baseline and at the end of the study. A total of 369 subjects completed at least one post-baseline visit. Of this population, 60 subjects had a baseline HbA1c ≥ 10% and were included in the post-hoc efficacy analysis.
Results: After 90 days, subjects in the active group had significant reduction in HbA1c level compared to placebo (-1.78% vs. -0.78%; P<0.004) as well as significant reduction in FPG levels compared to placebo (–35 mg/dL vs. +3.7 mg/dL, P<0.04). Mean reductions in HbA1c levels, grouped by antidiabetic medication were: (active vs. placebo); SU+B (-1.9 vs. -1.0); B (–1.9 vs. –1.1); TZD (-1.7 vs. -0.3); SU (-0.65 vs. +0.35). Insulin resistance (estimated using HOMA IR) was significantly reduced in subjects on TZD (P<0.05). Non-significant reductions in HOMA IR were seen in all other active groups.
Conclusion: The results of this study show that the addition of the CrPic and biotin combination can significantly improve glycemic control in poorly controlled subjects with type 2 diabetes mellitus.

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Chromium (Cr^{3+}) supplementation can prevent cellular glutathione (GSH) depletion and lipid peroxidation (LP) caused by ketosis by activating glutathione reductase (GR) in human red blood cells (RBC)
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Background and Aims: Type 1 diabetic patients frequently encounter ketosis. Type 1 diabetes is also associated with low cellular GSH and LP. GSH plays a pivotal role in the maintenance of redox balance and the cellular functions. The molecular mechanisms of GSH depletion and LP and how chromium supplementation is beneficial in preventing complications of diabetes is not known. Using RBC as a model, this study has examined the effect of trivalent chromium and acetoacetate (AA, a metabolite of ketosis in diabetes) and t-butylhydroperoxide (TBH, a standard oxidant) on GSH and LP levels.
Methods: Normal RBC (25% hematocrit suspended in PBS) were treated with AA (0–5 mM) or TBH (0–2 μM) with and without $CrCl_3$ in a shaking water bath at 37°C for 24 hrs.
Results: show a significant decrease in GSH and increase in LP both in AA (to 27%) and TBH (to 50%) treated RBC; and preincubation of RBC with $CrCl_3$ (0–1 μM) prevented the decrease in GSH and increase in LP in AA and TBH treated RBC. To delineate the mechanism, we determined that GR activity was 70–90% higher ($p<0.01$) in RBC with prior Cr-treatment compared with those without Cr, in cells incubated with AA or TBH.
Conclusion: GSH supplementation is known to improve insulin sensitivity, lower hyperglycemia and prevent complications in animal models of diabetes. This study demonstrates that Cr^{3+} supplementation can activate GR activity and prevent GSH depletion and cellular damage, and thus can be beneficial in preventing the complications of diabetes.
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Metabolic effects of high dose vitamin D treatment in type 2 diabetes patients
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Background and Aims: There is evidence for the preventive and immunmodulating effect of vitamin D in type 1 diabetes. However there are only few studies showing that vitamin D deficiency impairs the ability to produce insulin and respond to insulin, thus being involved in the outcome of type 2 diabetes too. The aim of our study was to determine the effect of vitamin D3 on carbohydrate metabolism and insulin resistance quantified by HOMA-IR in type 2 diabetes patients in the district of Mures, Romania.
Materials and Methods: Our study was a three-month randomised placebo controlled study. 72 type 2 diabetes patients were recruited and randomised to either Vitamin D3 1200 daily dose (39 patients, mean age 54,7 ± 12,1 years, HbA1c 7,6%) or placebo (33 patients, mean age 59,7 ± 10,5 years, HbA1c 7,3%). In each group basal insulinaemia (with conventional insulin assays), fasting glycaemia, HbA1c, calcium and magnesium level were assessed before and after the treatment. HOMA-IR was calculated with the formula: insulin (microU/ml) × glycaemia(mmol/l)/22,5. The trial included a control group consisting in 22 healthy adults.
Results: 71% of the studied population had hypocalcaemia (it was no significant difference between the groups). Results show marked and significant decrease in HbA1c (0,46%± 0,12%, p<0,05) and HOMA-IR (48,7%± 13,2% percentage decrease, p<0,01) in the vitamin D group versus placebo group (HbA1c 0,16%± 0,10%, HOMA -IR 7,8%± 4,3% percentage decrease). Calcium levels normalized in all vitamin D treated patients.

Conclusion: There is a significant hypocalcaemia in the adult population of our district. Vitamin D improves carbohydrate metabolism, and particularly insulin resistance in type 2 diabetes patients, at least in a population markedly hypocalcaemic.

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Influence of retinol supplementation on triglyceride and insulin metabolism in persons with different FABP2 promoter genotypes

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Background: Gene-variability in the Exon of the intestinal fatty acid binding protein (FABP2) is associated with a impaired insulin sensitivity und type-2-diabetes. Recently we and others have found a variability in the FABP2 promoter, which is associated with different HDL-levels and different atherogenic risc factors. In-silico analysis of the promoter have shown different binding sites for retinoid acid, depending on the variability of the promoter.
Aim: The influence of oral application of retinol on the fasting and postprandial triglyceride, glucose and insulin metabolism of carriers of different FABP2 promoter variants. Methods: Depending on gene variability of the FABP2 promoter, 40 subjects of the Metabolic Intervention Cohort Kiel (MICK) were included for an open labelled study. 20 subjects were carrier of the FABP2-promoter-variant A and 20 of the promoter variant B. 5000 I.E. Retinol per os were applicated for 8 weeks. Before and after intervention a metabolic tolerance test was performed.
Results: In-silico analysis showed different binding sites for retinoic acid depending on promoter polymorphism. Baseline-parameters of different genotype groups were not different at the beginning of retinol supplementation. After the period of 8 week of supplementation changes occured between groups for triglyceride fasting levels. Moreover postprandial insulin levels were changed, when stratified for FABP2 promoter genotypes, only difference in carriers of variant B reached significance. Free fatty acids were reduced in whole study cohort and stratification for FABP2-promoter leads to no difference between FABP2 promoter genotypes. Also changes in serum liver enzyme levels were found. GOT, GPT and CHE were elevated whereas γGT levels were influenced only weakly.
Conclusion: Retinol supplementation over a short period leads to changes in fasting triglyceride serum levels and postprandial insulin and free fatty acid serum levels. The triglyceride levels seam to be dependent on FABP2 promoter genotype. There are indication that changing in postprandial insulin levels might be influenced by FABP2 genotype whereas the postprandial free fatty acids are changed independent on FABP2 genotype.
Support: BMBF

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Impact of four-week fructose overfeeding on insulin sensitivity, tissular lipids and plasma triglycerides in healthy men

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Background and Aims: Over the past decades fructose consumption has dramatically increased, in parallel to the epidemic of overweight and obesity. Some authors suggest a causal relationship between fructose consumption, excess weight gain and the onset of metabolic disorders. In rodents, high fructose diet (HFr) induces after one week hepatic insulin resistance, hepatic de novo lipogenesis and high intrahepatocellular lipids (IHCL). When sustained over longer term, HFr impairs whole-body insulin sensitivity (IS) together with an accumulation of intramyocellular lipids (IMCL). In humans, the impact of HFr over several weeks remains unknown. This study was designed to evaluate the effects of a daily fructose consumption corresponding to the fructose content of ca 2 L soda beverage on plasma triglycerides (TG), glucose and lipid homeostasis. More specifically, it was aimed to investigate whether the sequential establishment of fructose-induced alterations (hepatic insulin resistance and high IHCL followed by whole body insulin resistance and high IMCL) observed in rodents was also found in humans.
Materials and Methods: Six healthy men underwent a four-week HFr (1.5 g fructose/kg bodyweight/day). Fasting blood samples were collected weekly, whereas insulin sensitivity and tissular lipid contents were measured before HFr, after one and four weeks of HFr. Hepatic and muscular IS were assessed by monitoring hepatic glucose production (HGP) and whole body glucose disposal rate (GDR) during a two-step hyperinsulinemic euglycemic clamp (0.3 and 1 mU/kg/min, 180 min each). HGP was evaluated using 6,6-2H2 glucose. Adipose tissue IS was assessed by measuring the decrease in systemic free fatty acids (FFA) during the low dose insulin clamp. IHCL and IMCL were measured by 1H magnetic resonance spectroscopy.
Results: HFr caused significant ($p < 0.05$) increase in fasting plasma TG (+ 60%, from 0.67 ± 0.21 to 1.10 ± 0.66 mmol/l) and glycemia (+ 9.3%, from 88.9 ± 7.2 to 95.5 ± 3.8 mg/dl) after one and two weeks, respectively. These concentrations remained increased throughout the HFr. Body weight, hepatic, muscular and adipose tissue IS as well as IHCL and IMCL remained unaffected by HFr (no difference between control, after one and four weeks of HFr), as indicated by unchanged suppression of HGP (76 ± 15, 79 ± 7 and 76 ± 7%, NS) and FFA (42 ± 9, 35 ± 19 and 46 ± 14%, NS) during the low dose insulin clamp, and unchanged whole body GDR (6.0 ± 0.6, 6.3 ± 0.5 and 6.2 ± 0.7 mg/kg/min, NS) during the high dose insulin clamp.
Conclusion: Fructose rapidly increased fasting TG and glycemia without any significant change in insulin sensitivity (hepatic, muscular and adipose) or IHCL / IMCL. This suggests that moderate fructose consumption causes hypertriglyceridemia (most likely due to stimulation of de novo lipogenesis), but is not sufficient alone to induce insulin resistance or alter IHCL and/or IMCL after four weeks. Healthy subjects may prevent the deleterious effects of fructose, possibly by increasing muscle oxidation of TG-rich lipoproteins. The development of fructose-induced insulin resistance and accumulation of IHCL / IMCL may however be accelerated in individuals presenting additional detrimental factors such as overweight and/or genetic predisposition to the metabolic syndrome.
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Natural sweetener stevioside improves lipid profile and ameliorates oxidative stress in diabetic rabbits

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Background and Aims: The Stevia rabaudiana Bertoni (SrB) plant has been used for many years in the traditional treatment of diabetes in Paraguay and Brazil. It has been shown that stevioside, a sweet-tasting diterpene glycoside isolated from SrB leaves, exerts antihyperglycaemic, insulionotropic, glucagonostatic and antihypertensive effects in type 2 diabetic rats. The aim of the study was to elucidate the impact of stevioside on lipid profile and oxidative stress in diabetic rabbits.
Materials and Methods: Male chinchilla rabbits were made diabetic by i.v. injection of dithizone (35 mg/kg b.w.). Control rabbits (C) were given vehicle alone. In a week after diabetes induction animals were randomised into two groups: one group was untreated to act as diabetic control (D) and other group received stevioside (0.2 g/kg/day per os) for a month. At the end of the study rabbits were subjected to the glucose tolerance test (GTT, 0.5 g/kg i.v.). Blood was sampled in a fasting state for analysis of glucose, plasma insulin, serum total cholesterol (TC), HDL-C, triglycerides (TG) and NEFA. Oxidative status of experimental animals was estimated by lipid peroxidation intermediates - plasma thiobarbituric acid reactive substances (TBARS) and total antioxidant activity (TAA).
Results: Administration of stevioside decreased basal hyperglycaemia (11.2 ± 0.5 vs D: 17.8 ± 0.9; C: 4.6 ± 0.2 mmol/l, $p<0.001$) and glucose intolerance (AUC over GTT was 787 ± 28 vs D: 1230 ± 70; C: 325 ± 45 mmol/l/min, $p<0.01$) compared to diabetic controls. Stevioside supplementation also ameliorated diabetic dyslipidaemia due to reduction in NEFA (0.66 ± 0.05 vs D: 1.59 ± 0.05; C: 0.36 ± 0.01 mmol/l, $p<0.001$), TG (0.69 ± 0.16 vs D: 4.88 ± 0.59; C: 0.72 ± 0.08 mmol/l, $p<0.001$) and TC (1.63 ± 0.05 vs D: 1.85 ± 0.09; C: 1.12 ± 0.03 mmol/l, $p<0.05$) levels. The treatment with stevioside elevated HDL-C by 1.5-fold ($p<0.01$) and reduced LDL-C by 35% ($p<0.05$) compared to D-group. Improvement of lipid profile in diabetic rabbits after stevioside administration was accompanied by decrease in TBARS concentration (1.67 ± 0.05 vs D: 3.15 ± 0.29; C: 1.12 ± 0.13 mmol/l, $p<0.01$) and increase in TAA by 32% ($p<0.05$) in comparison with control diabetic rabbits.
Conclusion: These data demonstrate that stevioside administration improves both glycaemic and lipid profiles, attenuates lipid peroxidation and enhances antioxidant defence in diabetic rabbits. We suggest that the use of natural sweetener stevioside may contribute not only to calorie reduction but also has additional benefits on cardiovascular complications development in type 2 diabetes.

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Novel thiazolidinediones and insulin sensitising agents

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Improvements in postprandial lipid handling and glucose tolerance with tesaglitazar in insulin-resistant, nondiabetic patients

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Background and Aims: Postprandial dyslipidaemia is associated with atherosclerosis and coronary heart disease and has been linked to insulin resistance. Postprandial hyperglycemia is an important risk marker for cardiovascular disease among patients with type 2 diabetes and for those with elevated glucose in the nondiabetic range. The present study assessed the effect of tesaglitazar (GALIDA™), a dual peroxisome proliferator-activated receptor (PPAR)α/γ agonist, on postprandial lipid handling and glucose tolerance in an insulin-resistant, nondiabetic population.
Materials and Methods: This investigation was part of the Study in Insulin Resistance (SIR; SH-SBT-0001), a randomized, double-blind, placebo-controlled clinical trial. SIR documented significant improvements in fasting lipid and glucose values after treatment with tesaglitazar (0.1, 0.25, 0.5, or 1.0 mg once daily for 12 weeks). A subgroup of 222 patients received a lipid-rich meal and/or an oral glucose tolerance test (OGTT) at baseline and treatment end. After the lipid-rich meal, placebo-corrected changes in the area under the concentration-time curve (AUC) were determined for serum triglycerides (TG), plasma free fatty acids (FFA), serum glycerol and plasma insulin. Two-hour plasma glucose levels were measured after the OGTT. Statistical analyses were performed using the intention-to-treat (ITT) population.
Results: Tesaglitazar improved postprandial lipid handling. AUC for TG was reduced after the meal in a dose-dependent manner (Table). Significant reductions were seen with tesaglitazar 0.25–1.0 mg, with a maximum reduction of 41% ($p<0.0001$) for tesaglitazar 1.0 mg. This dose also produced corresponding ($p<0.0001$) reductions in FFA, glycerol and insulin AUC (Table). Tesaglitazar 1.0 mg significantly improved the 2-hour plasma glucose levels during the OGTT, with a placebo-corrected reduction from baseline of -1.51 mmol/L ($p<0.01$). In addition, all patients in the tesaglitazar 1.0 mg group had normal glucose tolerance at treatment end compared with 85% at baseline.

Placebo-corrected changes from baseline (95% confidence limit)

Variable	Tesaglitazar 0.1 mg	Tesaglitazar 0.25 mg	Tesaglitazar 0.5 mg	Tesaglitazar 1.0 mg
Lipid-rich meal				
TG %	-12 (-25; -3)	-20** (-32; -6)	-30 (-40; -18)	-41 (-50; -30)
FFA %	-3 (-14; 9)	-11 (-22; 1)	-17** (-26; -5)	-29 (-38; -20)
Glycerol %	-7 (-21; 10)	-14 (-28; 2)	-19* (-32; -3)	-34 (-45; -22)
Insulin %	-5 (-21; 16)	-3 (-21; 18)	-16 (-32; 3)	-31 (-44; -15)
OGTT				
Glucose mmol/L	-0.22 (-1.08; 0.64)	0.10 (-0.77; 0.97)	-0.82 (-1.69; 0.05)	-1.51 (-2.42; -0.61)

*$p\leq0.01$; **$p<0.005$; $p<0.0001$

Conclusion: Tesaglitazar improved both postprandial lipid handling and glucose tolerance in insulin-resistant, nondiabetic patients in a dose-dependent manner. In particular, tesaglitazar 1.0 mg produced significant improvements in all variables examined. These effects may help reduce cardiovascular disease risk among insulin-resistant patients.
Study supported by AstraZeneca

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Tesaglitazar corrects glucose and lipid abnormalities in patients with type 2 diabetes

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Background and Aims: Tesaglitazar (GALIDA™) is a dual peroxisome proliferator-activated receptor (PPAR)α/γ agonist that improves glucose and lipid abnormalities in insulin-resistant, nondiabetic patients. The dose-finding study reported here examined the efficacy and safety of tesaglitazar in patients with type 2 diabetes.
Materials and Methods: Glucose and Lipid Assessment in Diabetes (GLAD; SH-SBD-0001) was a randomized, double-blind, placebo-controlled comparator study in patients with type 2 diabetes, defined as a fasting plasma glucose (FPG) ≥7.0 mmol/L. Male and female patients received tesaglitazar (0.1, 0.5, 1.0, 2.0 or 3.0 mg), placebo or open-label pioglitazone (45 mg), orally once daily for 12 weeks. End points included changes in FPG and lipid variables including triglycerides (TG), HDL-C, non-HDL-C, total C, LDL-C and VLDL-C. Fasting plasma insulin (FPI) and homeostasis model assessment in insulin resistance (HOMA-IR) were also examined. Statistical analyses were performed using the intention-to-treat (ITT) population.
Results: The ITT population included 485 patients. Mean (SD) baseline characteristics were similar between groups: HbA_{1c} 7.1 (1.0)%; FPG 9.4 (1.9) mmol/L; BMI 30.7 (4.7) kg/m^2. Tesaglitazar improved all glucose and lipid variables examined in a dose-dependent manner. Placebo-corrected reductions in FPG were significant for tesaglitazar doses ≥0.5 mg, with a maximum reduction of -60.9 mg/dL for tesaglitazar 3.0 mg (Table). Tesaglitazar dose-dependently improved TG, HDL-C, non-HDL-C, total-C, LDL-C and VLDL-C. Free fatty acid levels were also dose-dependently reduced by tesaglitazar. Significant, dose-dependent reductions in FPI (-27.3%, $p<0.05$) and HOMA-IR (-42.5%, $p<0.0001$) suggested decreased insulin resistance with tesaglitazar 1.0 mg. Changes from baseline in FPG were numerically similar for tesaglitazar 1.0 mg and pioglitazone 45 mg, while doses of tesaglitazar >0.5 mg produced numerically greater improvements in TG, HDL-C and non-HDL-C than pioglitazone 45 mg (Table). Tesaglitazar was well tolerated; adverse events were dose dependent. Rates of edema were similar among treatment groups (4.2-6.8% tesaglitazar vs. 4.2% pioglitazone 45 mg and 2.9% placebo) and there were no cases of heart failure.

Placebo-corrected changes from baseline (ITT population)

Variable	Tesaglitazar 0.1 mg	Tesaglitazar 0.5 mg	Tesaglitazar 1.0 mg	Tesaglitazar 2.0 mg	Tesaglitazar 3.0 mg	Pioglitazone 45.0 mg
n	71	72	69	69	69	68
FPG (mg/dL)	-8.9	-30.3‡	-41.1‡	-55.0‡	-60.9‡	-38.5
TG (%)	-5.4	-17.2*	-32.9‡	-41.0‡	-40.9‡	-8.2
HDL-C (%)	1.0	4.6	15.0‡	13.0	12.9‡	5.5
Non-HDL-C (%)	-3.2	-8.6*	-13.2‡	-22.2‡	-25.0‡	-2.5
Total C (%)	-2.6	-5.2	-6.8*	-14.1‡	-15.5‡	-0.6
LDL-C (%)	-2.9	-4.5	-6.4	-11.1*	-17.3‡	-4.6
VLDL-C (%)	-0.8	-15.1	-36.9‡	-49.8‡	-52.5‡	6.0

*$p<0.05$; $p<0.001$;‡$p<0.0001$
NB. No statistical comparisons were made between pioglitazone and other groups.

Conclusion: In conclusion, tesaglitazar produced dose-dependent improvements in glucose control and lipid abnormalities in patients with type 2 diabetes. Improving both glucose and lipid parameters may reduce the risk of microvascular and macrovascular complications in type 2 diabetes.
Study supported by AstraZeneca

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Glycemic efficacy and tolerability of muraglitazar, a novel dual (α/γ) PPAR activator, plus metformin in patients with type 2 diabetes and inadequate glycemic control

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Background and Aims: Muraglitazar (MURA), the first in a new class of drugs called glitazars, is a dual (α/γ) PPAR activator with both insulin-sen-

sitizing and lipid-regulating properties. The primary objective of this study was to compare the change in HbA_{1c} achieved with 2 doses of MURA plus metformin (MET) versus placebo (PLA) plus MET in subjects with type 2 diabetes and inadequate glycemic control on MET alone.
Materials and Methods: This double-blind, randomized, placebo-controlled study involved 652 patients with a baseline HbA_{1c} value of 7%-10% while on a MET dose of 1500–2550 mg/day. Patients received MURA 2.5 mg, MURA 5 mg, or PLA once a day for 24 weeks while continuing stable MET therapy. All analyses of change from baseline were adjusted for baseline level and used the LOCF methodology.
Results: The treatment groups were well balanced in demographics and entry characteristics; all groups had a mean HbA_{1c} of 8.0% at baseline. When combined with MET, both doses of MURA provided statistically significant (P<0.0001 vs. PLA) mean reductions in HbA_{1c} compared with PLA (**Table**). Mean HbA_{1c} values at week 24 in the MURA 2.5 mg and MURA 5 mg groups were 7.08% and 6.83%, respectively, corresponding to adjusted mean changes from baseline in HbA_{1c} of -0.91% and –1.16%, respectively. The incidences of adverse events (AEs) and serious AEs were similar for the MURA 2.5 mg, MURA 5 mg, and PLA groups. Discontinuation rates due to AEs were low in the MURA 2.5 mg, MURA 5 mg, and PLA groups. All reports of edema, except for one event in a patient receiving MURA 5 mg, were of mild/moderate intensity and did not require discontinuation of study medication. Modest weight gain was reported in the MURA 2.5 mg and MURA 5 mg groups. CHF occurred in 1 subject receiving MURA 5 mg; study drug was withdrawn and symptoms resolved within 24 hours.
Conclusion: The findings from this trial indicate that MURA in once-daily doses of 2.5 mg and 5 mg, taken with MET, was generally well-tolerated and substantially improved glycemic control in subjects with type 2 diabetes failing MET monotherapy**.**

CHANGE FROM BASELINE IN HbA1c AT WEEK 24 (LOCF)

PARAMETER	MURA 2.5mg + MET N = 233	MURA 5mg + MET N = 205	PLA + MET N = 214
Baseline Mean HbA_{1c} (%)	7.99	8.00	7.97
Adjusted Mean Change From Baseline (%)	–0.91	–1.16	–0.05
P-value (vs. PLA)	<0.0001	<0.0001	–

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Muraglitazar, a dual (α/γ PPAR activator, prevents onset of diabetes in pre-diabetic *db/db* mice, ameliorates diabetes in severely diabetic *db/db* mice, and preserves β-cell function in both mouse models
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Background and Aim: *db/db* mice, over time, develop obesity, severe insulin resistance, diabetes, glucosuria and progressive loss of β-cell function. The novel dual alpha/gamma PPAR activator muraglitazar was evaluated for its effects: 1) in preventing onset of diabetes in pre-diabetic *db/db* mice, 2) on diabetes in severely diabetic *db/db* mice and 3) in preservation of pancreatic β-cell function in both pre-diabetic and severely diabetic *db/db* mice.
Materials and Methods: Two separate studies were carried out. The first was a 12-week study with muraglitazar (10 mg/kg/day) in pre-diabetic *db/db* mice (~4–5 week old females), where fasting glucose and insulin levels were measured at 4, 8 and 12-weeks. The second was a 2-week study with muraglitazar (10 mg/kg/day) in severely diabetic *db/db* mice (~10–11 week old females); fasting glucose and insulin levels were measured at the end of the study. In both studies, oral glucose tolerance tests (oGTT) were performed and insulin content in isolated pancreas was determined at the end of treatment period
Results: In pre-diabetic *db/db* mouse study, muraglitazar treatment prevented the development of both hyperglycemia and hyperinsulinemia (decreases of 63%, 70% and 62% in fasted glucose levels and decreases of 31%, 43% and 54% in fasted insulin levels at the 4-, 8- and 12-week time points, respectively). At the end of the study, an oGTT was performed, which showed improved glucose tolerance (glucose AUC reduction of 43%). Pancreatic insulin levels in muraglitazar-treated mice were maintained at the levels normally observed for lean C57BL/6 mice (323% above vehicle treated group levels). In the severely diabetic *db/db* mouse study, muraglitazar treatment ameliorated both hyperglycemia (-51%) and hyperinsulinemia (-55%); glucose tolerance was improved, with a glucose AUC reduction of 30%. Furthermore, muraglitazar treatment increased pancreatic insulin content (380% above the vehicle treated group levels).
Conclusions: Muraglitazar treatment prevented the onset of diabetes in pre-diabetic *db/db* mice and ameliorated diabetes in severely diabetic *db/db* mice. In addition, increased pancreatic insulin content in the context of improved plasma glucose and plasma insulin levels suggests preservation of β-cell function in both pre-diabetic and severely diabetic *db/db* mice.

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Improvement of diabetic dyslipidemia with muraglitazar, a dual (α/γ) PPAR activator: a randomized, double-blind, placebo-controlled study in type 2 diabetes patients uncontrolled on metformin
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Background and Aims: Muraglitazar (MURA), a dual (α/γ) PPAR activator with insulin-sensitizing and lipid-regulating properties, is the first in a new class of drugs called glitazars. In early clinical trials, MURA improved glycemic control and affected key components (triglycerides [TGs] and high-density lipoprotein cholesterol [HDL-C]) of diabetic dyslipidemia. This study explored the lipid-regulating effects of MURA in type 2 diabetes patients with inadequate glycemic control on metformin (MET) alone.
Materials and Methods: A double-blind, randomized, placebo (PLA)-controlled, 24-week study was conducted in 652 patients with type 2 diabetes and HbA_{1c} 7%-10% on MET (1500–2550 mg/day). Patients were randomized to receive MURA 2.5 mg or 5 mg or PLA once daily while continuing a stable MET dose. Changes from baseline in fasting TG, HDL-C, apolipoprotein B (apoB), and non-HDL-C were assessed at weeks 12 and 24.
Results: Approximately 30% of patients in each group were receiving a statin at baseline and continued at a stable dose through week 12; after this time statin therapy could be added or increased as necessary to meet LDL-C goals. At week 12, significantly greater reductions in fasting TG levels were reported in the subjects randomized to MURA 2.5 mg or MURA 5 mg compared with PLA (both P<0.0001). Adjusted mean percent change in fasting TG from baseline was -13.88% for MURA 2.5 mg, -29.20% for MURA 5 mg, and +3.22% for PLA. In patients with baseline fasting TG levels ≥ 150 mg/dL, the adjusted mean percent change from baseline in TG was -19.70% and -34.84% in those receiving MURA 2.5 mg and MURA 5 mg, respectively, and -1.94% for those receiving PLA. Plasma levels of HDL-C increased and apoB decreased in the MURA-treated subjects relative to the PLA group (**Table**). At week 24, the percent changes from baseline in the MURA 2.5 mg, MURA 5 mg, and PLA groups, respectively, were: TG, -13.25%, -28.13%, and -1.04%; HDL-C, +8.47%, +14.82%, and +2.58%; apoB, -3.84%, -11.69%, and +0.48%; non-HDL-C, +3.94%, -2.45%, and +5.03%. All reports of edema, except for one event in a patient receiving MURA 5 mg, were of mild/moderate intensity and did not require discontinuation of study medication. Modest weight gain was reported in the MURA 2.5 mg and MURA 5 mg groups. CHF occurred in 1 subject receiving MURA 5 mg; study drug was withdrawn and symptoms resolved within 24 hours.
Conclusion: These data suggest that once-daily 2.5 mg and 5 mg doses of MURA, added to MET, resulted in substantial improvements in lipid profile in patients with type 2 diabetes who had poor glycemic control on MET alone.

CHANGE FROM BASELINE IN LIPID PARAMETERS ASSESSED AT WEEK 12

PARAMETER	MURA 2.5mg + MET N = 233	MURA 5mg + MET N = 205	PLA + MET N = 204
TG (mg/dL)			
Baseline Mean	197.79	208.02	197.01
Adj. Mean % Change From Baseline	–13.88	–29.20	+3.22
P-value	<0.0001	<0.0001	–
HDL-C (mg/dL)			
Baseline Mean	45.00	45.79	46.17
Adj. Mean % Change From Baseline	+8.00	+14.14	+0.95
Nominal P-value	<0.0001	<0.0001	–
apoB (mg/dL)			
Baseline Mean	99.64	100.01	98.90
Adj. Mean % Change From Baseline	–4.98	–11.91	+0.95
Non-HDL-C (mg/dL)			
Baseline Mean	144.95	145.56	143.05
Adj. Mean % Change From Baseline	+1.81	–4.56	+4.37

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BLX-1002, a new antidiabetic agent, inhibits cell respiration in isolated rat skeletal muscle: parallels with metformin, rosiglitazone, and pioglitazone

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Background and Aims: We have shown previously that metformin as well as the PPARγ-agonistic thiazolidinediones rosiglitazone and pioglitazone have direct effects on mitochondrial function. All these compounds impair cell respiration in isolated specimens of rat skeletal muscle, which is obviously due to a direct inhibition of complex 1 of the respiratory chain. BLX-1002 is a new agent with distinct insulin sensitising, antihyperglyceamic, and antihyperlipideamic action in various animal models of diabetes mellitus, but without any agonistic activity on any subtype of peroxisome proliferator-activated receptors (PPARs). To evaluate whether BLX-1002 also addresses the mitochondrial mechanism targeted by other insulin sensitising drugs, the present study examined the direct effects of BLX-1002 on cell respiration and mitochondrial function.
Materials and Methods: Activity of complex 1 of the respiratory chain was determined in muscle homogenates immediately after the addition of 10, 30, or 100 µmol/l BLX-1002. Furthermore, intact freshly isolated specimens of rat soleus muscle were exposed to BLX-1002 for 24 h, before rates of fuel metabolism were measured under insulin-stimulated (100 nmol/l) conditions during an additional hour of incubation. Experimental procedures were exactly as used in previous studies, which allowed for direct comparison of the effects of BLX-1002 to those of rosiglitazone, pioglitazone, and metformin. All data in this abstract are given as % change vs an intraindividual control muscle.
Results: BLX-1002 resembled the other antidiabetic drugs in that it markedly and dose-dependently inhibited the activity of respiratory complex 1 in homogenates of rat skeletal muscle (complex 1 activity: 10 µM BLX-1002, -8 ± 3%, p<0.05; 30 µM BLX-1002, -11 ± 2%, p<0.001; 100 µM BLX-1002, -38 ± 2%, p<0.001; 100 µM rosiglitazone, -54 ± 7%, p<0.001; 100 µM pioglitazone, -12 ± 4%, p<0.02; 30 mM metformin, -15 ± 2%, p<0.001). In line with inhibitory action on an enzyme complex essential for respiratory function, BLX-1002 impaired cell respiration in intact muscle specimens, as indicated by reduced oxidation of both glucose and fatty acids (CO_2 from glucose: 30 µM BLX-1002, +6 ± 7%, ns; 100 µM BLX-1002, -20 ± 4%, p<0.001; CO_2 from palmitate: 30 µM BLX-1002, +2 ± 7%, ns; 100 µM BLX-1002, -24 ± 8%, p<0.02), as well as by a distinct increase in lactate release (lactate release: 30 µM BLX-1002, +24 ± 11%, p<0.05; 100 µM BLX-1002, +62 ± 14%, p<0.001; 9 µM rosiglitazone, +38 ± 6%, p<0.002; 9 µM pioglitazone, +64 ± 11%, p<0.02; 270 µM metformin, +84 ± 12%, p<0.001). As a consequence of impaired cell respiration, exposure to BLX-1002 or the other antidiabetic agents caused a loss of cellular energy charge (ratio of PCr/ATP: 100 µM BLX-1002, -24 ± 6%, p<0.02; 27 µM rosiglitazone, -44 ± 6%, p<0.001; 27 µM pioglitazone, -37 ± 8%, p<0.001; 810 µM metformin, -43 ± 6%, p<0.002).
Conclusion: BLX-1002 resembles rosiglitazone, pioglitazone, and metformin in that it likewise inhibits respiratory complex 1 and impairs cell respiration of isolated skeletal muscle. A common mitochondrial mechanism of action could contribute to the antidiabetic effects of all these compounds, since the resulting cellular energy loss should activate AMP-activated protein kinase (AMPK) which is known to cause insulin sensitisation.

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Hypoglycemic effect of PE071 seeds in type 2 diabetic Long-Evens rats

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Background and Aims: From widely publicized media reports based on folkloric reputation thousands of diabetic patients in Bangladesh started to use the seed extracts of plant (coded as PE071) in recent times. We found no published result on the antidiabetic effects of the plant and decided to investigate the hypoglycemic effects and possible mode of action of the water extract of its seeds in type 1 and type 2 diabetic model rats.
Materials and Methods: Freeze-dried water extract was used for the study at a dose of 312.5 mg/kg body wt/10 ml of water. Male Long-Evans rats, bred at BIRDEM animal house and weighing between 180–200g were used in the study. Type 1 and 2 diabetic model of rats were produced with intraperitoneal injection of streptozotocin using conventional methods and following the procedure standardized in BIRDEM. Acute experiments were done in both the model rats with a single feeding in different prandial states. For chronic experiment type 2 diabetic rats were divided into 3 groups: i) Vehicle (receiving only water, n=7); ii) Treated with standard drug glibenclamide (5 mg/kg body wt, n=6) and iii) Treated with PE071 extract (once daily for 28 consecutive days, n=8). Blood was collected by cutting the tail tip at the beginning and middle (14th day) of the study period under mild ether anesthesia and by decapitation after 28 days. Pancreas and liver were taken out and processed for further investigations. The parameters assessed were serum glucose (by glucose-oxidase), serum insulin and total pancreatic insulin content (by ELISA), lipid profile (by enzymatic colorimetric) and liver glycogen (by Anthrone-reagent) method.
Results: Screening results for hypoglycemic activity of PE071 showed no effect in any prandial states of type 1 diabetic model rats. On the contrary, the extract significantly lowered serum glucose levels of type 2 diabetic rats in both the prandial states (simultaneous with oral glucose p=0.007 and 30 minutes prior to oral glucose p=0.001). Reduction in glucose level was comparable with glibenclamide treated group. Oral administration of the extract for 28 days resulted in a significant reduction in serum glucose level on the 14th days; serum glucose in mmol/l: 6.95 ± 1.75 in vehicle vs 4.91 ± 1.15 in PE071 treated group p=0.03 and on 28th days; 6.95 ± 1.20 vs 4.80 ± 0.51 p=0.002. Glibenclamide treated group ameliorated the diabetic condition to the same extent (on the 28th days p=0.001) in type 2 diabetic model rats. Fasting serum insulin and total pancreatic insulin content did not change in the PE071 treated or water control group. Glibenclamide treated group showed a 48% increase in serum insulin content. Liver glycogen content increased in the Extract and Glibenclamide treated groups, but the increase was not significant. Serum TG level decreased by 17% in the PE071 treated group whereas glibenclamide and water treated group showed no effect. Body weight did not change significantly in any of the groups during the treatment period.
Conclusion: Plant extract PE071 improves the glycemic status of type 2 diabetic model rats and it seems to act as an insulinomimetic and /or insulin sensitizing agent. The plant merits further exploration both chemically and biologically to identify the active principle(s) and mechanism of action.

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Mechanism of insulin secretory effects of traditional antidiabetic plant extract PE027

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Background and Aims: PE027 leaves have previously been shown to reduce blood glucose level in rats as well as humans. In the present study the effects of the ethanol extract and 5 partition fractions of the leave on insulin secretory mechanism were elucidated in isolated perfused pancreas, isolated islets and BRIN-BD11 cell line.
Materials and Methods: Extract PE027 and its 5 fractions were dissolved in Krebs-Ringer buffer containing 3 or 11 mM D-glucose and perfused through mesenteric and celiac vessels of whole pancreas. Pancreatic islets

were isolated with collagenase digestion from normal Long-Evans rat. Batches of 8–10 islets were incubated for 1 hour at 37°C in Ca5 buffer containing either 3 or 11 mM of glucose with or without extract/fractions (30 µg/ml). Acute insulin release from clonal BRIN-BD11 cells was determined using cell monolayers. Cells were incubated for 20 min in KRB supplemented with different concentrations of extract and fractions. For the studies on the mechanism underlying the insulin secretory activity of extract/fractions, cells were incubated for 20 min in KRB in the presence of non-toxic concentrations of extract/fractions with or without 16.7 mM D-glucose, 50 µM verapamil, 300 µM diazoxide, 100 µM IBMX, 200 µM tolbutamide and 16.7 mM D-glucose plus 30 mM KCl. Intracellular calcium ($[Ca^{2+}]_i$) was determined in the monolayers of BRIN-BD11 cells using FLEX calcium assay kit in the FLEXstationTM, a scanning fluorometer and integrated fluid transfer workstation (Molecular Device, CA, USA).
Results: The ethanol extract PE027 and its 3 partition fractions (aqueous, butanol and ethylacetate) stimulated insulin secretion in isolated perfused rat pancreas and isolated rat islets. The effect of insulin secretion was further enhanced in the presence of 11 mM glucose in both the experiments. In acute, 20 min incubation, they evoked a stepwise enhancement of insulin secretion from BRIN-BD11 cells. The stimulatory effect of the ethanol extract (200 µg/ml) and 3 fractions (aqueous, butanol and ethylacetate; 1000 µg/ml) was potentiated by glucose, IBMX, tolbutamide and deporalizing concentration of KCl and inhibited in the presence of diazoxide and verapamil. The stimulatory effect of chloroform (1000 µg/ml) and hexane (1000 µg/ml) was unaltered by the presence of diazoxide and verapamil. The acute incubation (10 min) of BRIN-BD11 cells with extract and 5 fractions induced intracellular Ca^{2+}secretion. These effects were attenuated by the addition of verapamil. Toxicity testing of insulin releasing concentrations of these extracts, as evaluated by modified neutral red assay on BRIN-BD11 cells revealed that their action could not be attributed to a mere lysis of the cells, indicating that their secretory activity is mediated through physiological pathways.
Conclusion: The constituents of PE027 have wide-ranging stimulatory effects on physiological and pharmacological insulinotropic pathways.

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Antidiabetic effects of plant extract PE052 on type 1 diabetic model rats
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Background and Aims: Background studies in BIRDEM have shown that plant extract PE052 from the leaves of a medicinal plant have hypoglycemic activity in type 1 diabetic model rats. The present study was undertaken to explore the mechanism of its hypoglycemic activity, specifically to differentiate its protective or regenerative effect on pancreatic islet mass and function.
Materials and Methods: The dried powder leaves of PE052 were extracted with 80% ethanol. The rats were made type 1 diabetic by intraperineal injection of streptozotocin. The rats were fed with ethanol extract at a dose of 1.25 gm/kg body weight/10 ml water once a day for 14 days in 3 groups: PE052-0 group (n=9) was fed with the extract immediately, PE052-3 (n=7) was fed from 3rd day and PE052-5 (n=10) from 5th day after injection of streptozotocin. The water control (WC) group (n=17) was fed only with equal volume of deionized water for 14 days. Blood samples were drawn by cutting the tail tip at baseline and after 7 days of feeding. After 14 days the rats were sacrificed and blood was collected from heart; pancreas and liver were taken out and processed for further investigation. Isolation of islets was done by collagenase digestion method. Total pancreatic insulin content was measured by following standard technique. Serum glucose was measured by glucose oxidase method, serum insulin and total pancreatic insulin content by ELISA method. Liver glycogen was estimated following anthrone method.
Results: Fasting serum glucose of PE052-3 was significantly lowered 7 days as compared to the control (serum glucose in mmol/l: 15.5 ± 4.9 in WC vs 6.4 ± 1.4 in PE052-3, M±SD, p=0.003) and all PE052 treated groups showed significantly lower glucose level than the control at 14 days (Serum glucose in mmol/l: 17.6 ± 5.1 in WC vs 11.0 ± 4.3 in PE052-0 vs 8.4 ± 4.1 in PE052-3 vs 10.4 ± 5.6 in PE052-5, M±SD, p=0.012, p=0.006, p=0.033 respectively). Body weight of all rats (both control and treated) was significantly decreased from their initial weight within the group (p=0.001). The islet morphology was better in the treated group (PE052-0) when compared to the control in islet isolation study but no significant difference was observed in the number of islets. Fasting serum insulin level, total pancreatic insulin content and liver glycogen revealed no significant change between the treated and the control group.
Conclusion: The hypoglycemic effect plant extract PE052 in Type 1 diabetic rats is mediated by insulin sensitizing and/or insulinomimetic action and the extract may play a protective role against streptozocin induced islet damage.

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Metformin and thiazolidinediones: therapeutic effects

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Endothelial function and oxidative stress are changed by metformin in type 2 diabetes

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Background and Aims: Vascular effects of metformin have been supposed. Aim of this study was to evaluate the effect of metformin on oxidative stress, biochemical indicators of endothelial activity and microcirculation in type 2 diabetic patients.
Patients and Methods: In a pilot cross-over, placebo-controlled study fifteen type 2 diabetic patients (mean age 55 yrs, range 44–68 yrs) were treated with metformin (2×850 mg daily) or placebo during 3 months. Plasma malondialdehyde (MDA), nitrites/nitrates, alpha-tocopherol and ascorbic acid concentration and superoxide dismutase (SOD) activity in erythrocytes were determined before and after 1 and 3 months of the respective treatment. Serum E-selectin, intercellular cell adhesion molecules (ICAM-1), vascular cell adhesion molecules (VCAM-1), vascular endothelial growth factor (VEGF), von Willebrand factor (vWF) and tissue plasminogen activator (tPA) were estimated as indicators of endothelial activity. Skin microcirculation in the forearm was performed by laser-Doppler flowmetry.
Results: Metformin caused a borderline decrease of glycated hemoglobin HbA_{1c} after 3 months (8.3 ± 0.9 vs 8.1 ± 1.0%, $p<0.05$). Significant increase of plasma MDA (1.87 ± 0.47 vs 2.36 ± 0.27 μmol/l, $p<0.001$) and serum ascorbic acid (58 ± 12 vs 73 ± 19 mg/l, $p<0.001$) concentrations as well as of alpha-tocopherol ($p<0.01$) after 1 and 3 months of treatment were observed. SOD activity was increased only after 1 month (0.74 ± 0,37 vs 0,89 ± 0,36 U, $p<0.05$) with the following decrease to the initial values after 3 months of treatment. Serum nitrites/nitrates were decreased after 3 months of metformin administration (24.0 ± 11,6 vs 19.9 ± 7.2 μmol/l, $p<0.05$). Similarly, plasma tPA and serum concentration of ICAM-1, VCAM-1 and VEGF were significantly decreased after 3 months of metformin administration (6.9 ± 1.9 vs 5.6 ± 1.8 ng/ml, $p<0.02$, 257 ± 54 vs 222 ± 44 μg/l, $p<0.001$, 874 ± 161 vs 740 ± 133 μg/l, $p<0.001$, and 286 ± 154 vs 263 ± 144 μg/l, $p<0.02$). vWF was increased after 1 month of metformin treatment (106 ± 11 vs 112 ± 8%, $p<0.05$) and then its was decreased to the initial value. No relationship between variables of oxidative stress or cell adhesion molecules and diabetes control were found. Significant relationship was observed between 3 month changes of nitrites/nitrates and ICAM-1 ($r=0.39$, $p<0.05$) or VCAM-1 ($r=0.43$, $p<0.02$) concentrations, respectively. Microcirculation measured by laser-Doppler flowmetry using postocclusive or thermal hyperemia was not changed by metformin administration.
Conclusion: We conclude that metformin treatment activates oxidative stress together with antioxidant system. This is accompanied by changes in nitric oxide availability. The endothelial activity is therefore modified as it could be observed from diminished cell adhesion molecule and tPA concentrations. Our results demonstrate that metformin promotes endothelium-related effects in type 2 diabetes and protective role in development of endothelial dysfunction may be supposed after longer administration of the drug.
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Comparative metabolic effects of a novel fructose 1,6-bisphosphatase inhibitor and metformin in the female ZDF rat

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Background and Aims: Excessive glucose production via hepatic gluconeogenesis (GNG) is an important contributor to fasting and postprandial hyperglycemia in type 2 diabetes (T2DM). MB06322 (CS-917) is a prodrug of a rationally-designed, specific inhibitor (MB05032) of a key enzyme in GNG, fructose 1,6-bisphosphatase (FBPase). MB06322 markedly inhibits GNG, as measured by the D_2O tracer technique, as well as lowers blood glucose in male Zucker Diabetic (ZDF) rats. Metformin is believed to lower blood glucose in patients in part by inhibiting GNG via an unknown and indirect mechanism. The female ZDF rat, like its male counterpart, has a leptin receptor defect and is consequently hyperphagic, obese, and insulin resistant. Unlike the males, females only develop hyperglycemia when fed a high fat diet, and progress more slowly to overt diabetes and pancreatic failure. The purpose of these studies was to compare the efficacy and metabolic side effects of metformin and MB06322 in this fat-induced diabetic model.
Materials and Methods: Female ZDF rats (n=8/group) were placed on a diet containing 48% kcal fat (Research Diets 13004) for ~30 days, divided into glucose-matched groups and administered MB06322 (0, 30, 100 mg/kg) or metformin (30, 100, 300 mg/kg) for up to 21 days. Food and water intake, body weight, and all key blood/plasma parameters were measured at regular intervals. An oral glucose tolerance (OGT) test was performed after 16 days of treatment. Livers were harvested at sacrifice for triglyceride and cholesterol determinations. In a second study, the expression level of key GNG enzymes was measured by RT-PCR in livers of animals treated with MB06322 at 100 and 300 mg/kg for 14 days.
Results: MB06322 lowered blood glucose in a dose-dependent manner. On day 21, mean (±SEM) blood glucose levels were 361 ± 36, 285 ± 53, and 221 ± 35 mg/dL for controls and MB06322 at 30 and 100 mg/kg, respectively ($p<0.05$). Metformin was inefficacious at 30 mg/kg, but at 100 and 300 mg/kg lowered blood glucose to 290 ± 36 and 136 ± 13 mg/dL, respectively. Neither drug altered steady state levels of blood lactate, plasma triglycerides, plasma insulin, plasma ketone bodies, or liver triglycerides and cholesterol. OGT following a 6-h fast was not affected by either drug treatment. At the highest dose evaluated (300 mg/kg), metformin-treated animals consumed significantly less food than controls (29%, $p<0.05$) which may have contributed to the improvement in glycemia. MB06322 treatment at 300 mg/kg increased the transcription of the phosphoenolpyruvate carboxykinase, FBPase, and glucose 6-phosphatase genes. This dose was nevertheless similarly efficacious to 100 mg/kg at which these changes were not observed. Weight gain was unaltered by metformin or MB06322.
Conclusion: Both MB06322 and metformin lowered blood glucose levels in the female ZDF rat without evidence of metabolic perturbation, hypoglycemia, or weight gain. Insulin sensitivity was not improved during the relatively short treatment period. Despite the apparent compensatory upregulation of GNG enzymes at the highest dose of MB06322 evaluated, a waning in efficacy was not observed. Metformin caused significant decreases in food intake, potentially as a consequence of gastrointestinal disturbances. MB06322, in contrast, was well-tolerated. MB06322 may provide an effective treatment of T2DM, however, its safety and efficacy have not been proven in human clinical trials.

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Rosiglitazone and metformin fixed-dose combination provides superior glycaemic control compared to metformin and rosiglitazone monotherapies, and was well tolerated in drug-naive type 2 diabetes patients

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Background and Aims: International and US guidelines developed for the management of glycaemia in type 2 diabetes (T2DM) target normal blood glucose levels. Despite the benefits associated with tight glycaemic control in reduction of diabetes related complications and mortality, intensive intervention with combination therapy is often not used as initial therapy. Rosiglitazone (RSG) and metformin (MET) lower glucose concentrations by different mechanisms of action, and when used in fixed-dose combination (RSG/MET), offer therapeutic advantage for control of glycaemia. In this randomised, double-blind study, the efficacy and safety of RSG/MET was compared to RSG and MET monotherapies as initial therapy in drug-naive patients with T2DM.
Materials and Methods: Drug-naive subjects, 18–70 years of age, with a clinical diagnosis of T2DM were screened over a 2-week period. Eligible subjects ($HbA_{1c} \geq 7.5\%$ and $< 11\%$ and fasting plasma glucose [FPG] <15 mmol/l) received 32 weeks of blinded treatment with RSG/MET, RSG or MET. RSG/MET was initiated at 2 mg/500 mg and could be titrated to 8 mg/2000 mg in increments of 2 mg/500 mg. RSG was initiated at 4 mg and could be titrated to 8 mg. MET was initiated at a dose of 500 mg and could be titrated to 2000 mg in increments of 500 mg. Double-blind medication was titrated based on a rigorous glycaemic target of mean daily glucose ≤6.1 mmol/l.
Results: Mean baseline HbA_{1c} and FPG were comparable between treatment groups (8.8–8.9% and 10.8–11.2 mmol/l, respectively). Significant reduction in HbA_{1c} was observed with RSG/MET (-2.3%) compared with RSG (-1.6%) and MET (-1.8%); treatment differences for RSG/MET com-

pared with RSG and MET were -0.6% (P <0.0001) and -0.4% (P <0.001), respectively. Within 4 weeks, FPG declined 2.1 mmol/l with RSG/MET compared with RSG (-1.6 mmol/l) and MET (-1.2 mmol/l). After 32 weeks of treatment, RSG/MET significantly reduced FPG (-4.1 mmol/l) compared with RSG (-2.8 mmol/l) and MET (-2.8 mmol/l). More patients reached HbA_{1c} targets of ≤6.5% and <7% with RSG/MET (60% and 77%) compared to RSG (36% and 58%) and MET (39% and 57%), respectively. Weight gain with RSG/MET was negligible with a mean change from baseline (±SE) of 0.0 ± 0.5 kg compared to 1.5 ± 0.5 kg with RSG and -2.9 ± 0.4 kg with MET. The most frequently reported adverse events were gastrointestinal side effects of nausea/vomiting (16% RSG/MET; 13% MET, 8% RSG) and diarrhoea (14% RSG/MET; 21% MET, 11% RSG). The number of subjects with hypoglycaemia documented by symptoms and a fingerstick blood glucose measurement ≤ 2.8 mmol/l was low (1 RSG/MET, 3 MET, 0 RSG). Rates of oedema were comparable between RSG/MET and RSG and no events of congestive heart failure were reported.
Conclusion: As initial therapy in patients with T2DM, RSG/MET was superior to MET and RSG in lowering HbA_{1c} and FPG, and was generally well tolerated with no new safety or tolerability issues identified.

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Early combination therapy with pioglitazone reveals the highest benefits in patients with type 2 diabetes mellitus
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Background and Aims: Type-2-Diabetes mellitus (T2DM) is a disease with progressively deteriorating glycaemic control. Insulin resistance (IR) and interrelated declining betacell function (BCF) are believed to be the core defects of the disease. Pioglitazone (PIO) is a PPARγ-agonist offering unique and sustained glucose-lowering benefits via ameliorating IR and BCF. In addition PIO specifically targets vascular IR, most possible via reduced endothelial dysfunction and inflammation. Due to its mode of action PIO requires a sufficient amount of endogenous insulin to ameliorate metabolic control. Preliminary evidence suggest that reduction in HbA_{1C} is related to baseline values and remaining BCF. Data is lacking whether the anti-inflammatory effects of PIO are independent of its metabolic actions. Therefore we studied the glycaemic response and the anti-inflammatory effects of PIO in a cohort of rather unselected patients with T2DM.
Materials and Methods: Open-label, non-controlled observational study performed according to the guidelines of the German Federal Health Ministry. 1,250 patients with T2DM with a stable monotherapy of Metformin (MET) with a mean dose of 1,900 mg/day were recruited. Combination therapy with PIO 30 mg qd. was initiated for a mean observation time of 24 ± 2 weeks. PIO and MET dosage were kept stable throughout the observation period. Primary parameters were the changes of HbA_{1C} and high sensitivity C-Reactive Protein (hsCRP) compared to that at Baseline using the last observation carried forward (LOCF) approach. Multivariate analyses for covariables of treatment response were performed. For a subgroup of patients with available hsCRP measurements (n = 552), reductions were plotted against quartiles of HbA_{1C} reductions. All results are expressed as means±SE.
Results: HbA_{1C} declined from 8.5%± 1.1 to 7.2%± 1.2 ($p < 0.005$); hsCRP was reduced by 35% (3.92 ± 3.12 mg/l to 2.55 ± 2.66 mg/l, $p < 0.001$). Only HbA_{1C} at BL ($r = 0.55$) and diabetes duration ($r = 0.34$) showed a correlation to HbA_{1C} response, whereas age, gender, blood pressure, WHR, hsCRP and blood lipids did not. The results for changes of HbA_{1C} according to quartiles of HbA_{1C} at BL as well as for known diabetes duration were calculated.

Parameter	Q1	Q2	Q3	Q4
Diabetes Duration (years)	0.2–2.8	2.81–5.5	5.5–8.2	8.2–11.5
HbA1C Reduction (%)	–1.9	–1.4	–1.3	–1.0
HbA1C at BL (%)	5.6–7.0	7.01–8.3	8.31- 9.6	9.6–11.6
HbA1C Reduction (%)	– 0.8	–1.0	–1.4	–1.7

For patients with the shortest diabetes duration (Q1) and HbA_{1C} values at BL in Q3-4 the mean reduction compared to that at BL was - 2.26 ± 1.6% ($p < 0.001$ vs. BL). There was no significant difference for reduced hsCRP values in different HbA_{1C} quartiles at BL: -37%, -42%, -31% and -34% in Q1-4, respectively.
Conclusion: PIO revealed significant benefits for glycaemic control, especially when used as early combination therapy. In contrast, the anti-inflammatory effects of PIO were observed independently of glycaemic response.
Support: Takeda Pharma

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Randomized controlled trial of the effect of rosiglitazone in combination therapy on ambulatory blood pressure in people with type 2 diabetes mellitus followed for 12 months
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Background and Aims: Rosiglitazone improves blood glucose control through improving insulin sensitivity, and ameliorates a number of cardiovascular risk factors, in people with type 2 diabetes (T2DM). Rosiglitazone might also have an antihypertensive effect, but the size of this effect and how it is sustained in the medium term are uncertain. A sub-study of the RECORD trial has therefore been performed in people with T2DM, evaluating the effect of rosiglitazone in combination with other glucose-lowering therapies on ambulatory blood pressure (ABPM) for a period of 12 months.
Materials and Methods: At baseline, people inadequately controlled on metformin (n=379) or a sulfonylurea (n=380) were formally randomized to add-on rosiglitazone vs sulfonylurea, or rosiglitazone vs metformin, respectively. 24-hour ABPM was performed at baseline, and at 6 and 12 months. Analysable ABPM data was available in 668 people (160–176 in each treatment group) at 12 months. Baseline characteristics were comparable between treatment groups within stratum (including other cardiovascular medication); >80% of participants had hypertension at baseline.
Results: Change in the use of other anti-hypertensive medications was consistent across treatment groups. At 12 months, rosiglitazone with metformin lowered systolic BP by 4.8 ± 0.9 (SE) mmHg, compared to 2.1 ± 0.9 mmHg for sulfonylurea with metformin (difference 2.7 [95% CI 4.8, 0.7] mmHg, p=0.009), and diastolic BP by 3.7 ± 0.5 vs 1.7 ± 0.6 mmHg (difference 2.0 [3.3, 0.8] mmHg, p=0.002). Rosiglitazone with sulfonylurea lowered systolic BP by 3.8 ± 1.0 mmHg compared to 1.1 ± 1.0 mmHg for metformin with sulfonylurea (difference 2.6 [4.9, 0.4] mmHg, p=0.02), and diastolic BP by 3.5 ± 0.6 mmHg vs 0.4 ± 0.6 mmHg (difference 3.2 [4.5, 1.9] mmHg, p<0.001).
Conclusion: Combination rosiglitazone therapy, when compared to metformin and sulfonylurea in combination, produces sustained reductions in blood pressure in people with type 2 diabetes.

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Pioglitazone modifies not only distribution but also density of adipose tissue in type 2 diabetic patients
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Background and Aims: Pioglitazone, a thiazolidine-dione derivative, has been shown to induce the shift of intraabdominal fat to subcutaneous fat, indicating improvement of sick fat cells and thus amelioration of glucose metabolism in type 2 diabetic patients. However, previous reports from Japan failed to demonstrate the decrease of the intraabdominal fat area. We hypothesized the improvement of qualities of adipose tissue even in the intraabdominal space, and to demonstrate this improvement we examined the fat density in addition to the area and plasma adiponectin and TNF-α concentration before and after treatment of pioglitazone administration in Japanese type 2 diabetic patients.
Materials and Methods: Twenty-one type 2 diabetic patients (M/F=11/10, Age=63 ± 8 y.o., BMI=25.8 ± 0.6 kg/m^2) received abdominal CT examinations before and 12 months after the start of administration of pioglitazone. Abdominal CT examinations (GE Hilight Advantage) were conducted at the level of L_{4-5} and at the level of the liver and spleen. Image files were analyzed by ImageJ (NIH) after conversion of the original images into images with selected density (840 _ 975 pixels, W=400, L=0) to determine the area and histograms of the distribution of CT values, which were expressed by Housfield numbers, to identify the detailed composition of abdominal tissues and the liver. Plasma adiponectin and TNF-α levels were determined by ELISA.
Results: HbA_{1C}, plasma adiponectin and TNF-α concentration improved from 7.5 ± 0.9 to 6.8 ± 0.8%, 6.9 ± 3.2 to 15.7 ± 6.9 μg/ml, and 2.6 ± 1.2 to 0.8 ± 0.5 pg/ml respectively ~12 months after the start of administration of pioglitazone (22.6 ± 6.5 mg/day). There was no correlation between the change of HbA_{1C} and the change of adiponectin concentrations. The ratio of CT values of the liver and the spleen (L/Sp) was improved from 1.11 ± 0.17 to 1.23 ± 0.16 ($p<0.05$). The intraabdominal and subcutaneous fat area was changed from 149 ± 16 to 151 ± 21, and 174 ± 21 to 222 ± 35 cm^2

respectively. Average CT value in the intraabdominal fat area was decreased from -95.7 ± 17.2 to -113.5 ± 22.9 ($p<0.05$). The change of intraabdominal fat density was related to the change of TNF-α concentration.
Conclusion: It is possible to interpret that the significant change of intraabdominal CT value represents the affected intraabdominal fat tissue by the administration of pioglitazone, and the increase of L/Sp represents reduction of fatty accumulation in the liver. Thus, the *in vivo* effect of pioglitazone resides not only in the shift of the distribution of adipose tissue but also in the change of quality in the intraabdominal fat tissues, reflecting improvement of sick fat cells even in the intraabdominal space and resulting improved constitution of plasma adipocytokines. This consequence of pioglitazone administration should benefit preventing vascular complications of type 2 diabetes mellitus.

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Role of pioglitazone (PIO) in patients with non-alcoholic steatohepatitis (NASH)
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Background and Aims: patients with NASH are often insulin resistant and predisposed to develop type 2 diabetes. Thiazolidinediones (TZDs) reduce hepatic fat content and improve insulin sensitivity, but studies using TZDs in NASH have been limited to uncontrolled trials. We have performed the first randomized, double-blind, placebo (Pbo)-controlled trial to examine the role of PIO in NASH.
Materials and Methods: We enrolled 52 IGT/T2DM patients with biopsy proven NASH. Subjects received PIO (45 mg/d) or Pbo for 6 months and underwent the following plasma measurements and metabolic studies before and after treatment: (i) biochemical parameters (HbA_{1c}, ALT/AST levels, FFA, adiponectin, inflammatory markers, etc.); (ii) magnetic resonance spectroscopy (MRS) to measure liver fat; (iii) oral glucose tolerance test (OGTT) with the use of a double tracer technique ($^{14}C/^{3}H$ glucose) to assess glucose tolerance and hepatic/peripheral glucose metabolism; (iv) liver biopsy.
Results: We report on the metabolic data in the first 40 patients (PIO: 22; Pbo: 18). Two patients dropped out in each arm. Baseline characteristics were similar in PIO/Pbo. Findings: (i) metabolic control: PIO improved HbA_{1c} (6.0 ± 0.3% vs. 5.3 ± 0.1%, $p<0.004$) and decreased plasma FFA by 25% ($p<0.01$) despite a trend to increase BMI (33.0 ± 1.1 to 34.4 ± 1.3 kg/m^2, $p<0.08$ vs. pretreatment; NS vs. Pbo). ALT/AST levels decreased in all patients on PIO ($p<0.001$ vs. pretreatment and Pbo). No differences in any metabolic parameter occurred with Pbo; (ii) hepatic fat by MRS: PIO reduced liver fat content by 50% (22 ± 4% vs. 11 ± 3%, $p<0.005$; $p<0.05$ vs. Pbo); (iii) OGTT: PIO significantly reduced mean plasma glucose (-18%, $p<0.002$) and insulin (-25%, $p<0.05$) concentrations; (iv) Histologic analysis of liver biopsies (n=32, ADA 2005): the combined pathological score (hepatocellular ballooning, necrosis, lobular inflammation, macrovesicular steatosis, etc.) was significantly improved only with PIO ($p<0.0001$; $p<0.03$ vs. Pbo). PIO increased adiponectin by ~2.5-fold ($p<0.01$ vs. Pbo) and this correlated with an improvement in liver histology (necrosis: r=0.66; necroinflamm: r=0.67; % area fat: r=0.74; all $p<0.05$). Inflammatory markers also decreased with PIO: CRP by ~50% ($p<0.05$) while VCAM ($p<0.01$) and ICAM ($p<0.05$) by ~10%.
Conclusion: PIO treatment for 6 months in pts with NASH significantly improves multiple metabolic and histologic abnormalities. These results may have significant clinical implications for the future management of patients with NASH.

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Effects of PPAR-alpha and PPAR-gamma agonists on muscle AMPK activity and insulin resistance in patients with type 2 diabetes mellitus
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Background and Aims: The effect of PPAR-alpha and PPAR-gamma agonists on skeletal muscle AMP-Activated Protein Kinase (AMPK) activity has not been examined in patients with type 2 diabetes. To examine the effects of pioglitazone (PPAR-gamma) and fenofibrate (PPAR-alpha) on skeletal muscle AMP-Activated Protein Kinase activity, 6 type 2 diabetic patients (age = 51 ± 3 y, BMI = 32.5 ± 1.3, HbA_{1c} = 9.2 ± 0.7%) received fenofibrate (200 mg/d) for 3 months followed by the addition of pioglitazone (45 mg/d) for 3 months.
Materials and Methods: Subjects received 4-hour euglycemic insulin (80 mU/m^2-min) clamp with 3-^{3}H-glucose and vastus lateralis muscle biopsies at 0, 3, 6 months.
Results: After fenofibrate monotherapy, fasting plasma glucose (193 ± 20 to 189 ± 35 mg/dl), HbA_{1c} (9.2 ± 0.9 to 8.8 ± 0.8%), whole body insulin-mediated glucose disposal (Rd) (4.1 ± 0.8 to 4.5 ± 0.8 mg/kg-min) and plasma adiponectin concentration (3.5 ± 0.2 to 3.4 ± 0.3 μg/ml) did not change; plasma triglyceride concentration decreased from 206 ± 19 to 136 ± 21 mg/dl ($p<0.05$). Following fenofibrate monotherapy, muscle AMPK activity (phosphorylated AMPK to AMPK ratio) did not change (1.2 ± 0.2 to 1.2 ± 0.2). Addition of pioglitazone to fenofibrate increased Rd (4.5 ± 0.8 to 6.5 ± 0.4 mg/kg-min, $p<0.05$) and plasma adiponectin concentration (3.4 ± 0.3 to 11.4 ± 2.5 μg/ml, $p<0.05$), while fasting plasma glucose (189 ± 35 to 129 ± 12 mg/dl, $p<0.05$), HbA_{1c} (8.8 ± 0.8 to 7.3 ± 0.7%, $p<0.05$), and plasma triglyceride concentration (136 ± 21 to 88 ± 10 mg/dl, $p<0.05$) decreased. Following the addition of pioglitazone, muscle AMPK activity increased almost two-fold (1.2 ± 0.2 to 2.2 ± 0.3, $p<0.01$). Taken collectively before and after the addition of pioglitazone, muscle AMPK activity was positively correlated with plasma adiponectin concentration ($r=0.72$, $p<0.01$) and Rd ($r=0.49$, $p<0.10$).
Conclusion: Treatment of type 2 diabetic patients with a PPAR-gamma agonist (pioglitazone) improves glycemic control, decreases plasma triglyceride concentration, enhances insulin sensitivity, and increases muscle AMPK activity in association with an increase in plasma adiponectin concentration. The increase in AMPK activity is positively correlated with the increase in plasma adiponectin concentration and enhancement in insulin sensitivity. Treatment with a PPAR-alpha agonist (fenofibrate) has no effect on plasma adiponectin concentration, insulin sensitivity or muscle AMPK activity in patients with type 2 diabetes, despite a reduction in plasma triglyceride levels.
Support: National Institutes of Health

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Treatment satisfaction with early combination sulfonylurea (SU) and rosiglitazone (RSG): the RESULT trial
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Background and Aims: Treatment satisfaction may improve medication adherence which leads to long-term glycemic control. This study compared treatment satisfaction for elderly patients with type 2 diabetes (T2D) randomized to progressively uptitrated SU monotherapy with glipizide (n=110) or early addition of RSG to submaximal SU (n=114) in the RESULT trial, a 2-year double-blind, placebo-controlled trial.
Materials and Methods: The Diabetes Treatment Satisfaction Questionnaire (DTSQ) was used to measure patient satisfaction with treatment (range 0–36), perceived fear of hyperglycemia (range 0–6) and hypoglycemia (range 0–6). Treatment was individualized, targeting ADA defined goals, as appropriate, with uptitration required for FPG>180 mg/dL to a max of glipizide 20 mg bid and RSG 4 mg bid.
Results: At study end, RSG+SU group was significantly more satisfied with treatment as compared to SU group [Mean(SD); 33.1(3.49) vs 30.7(6.82), $p<0.001$]. Compared to baseline, RSG+SU group experienced a significant increase in treatment satisfaction [+1.4(4.78)], while SU group experienced a significant decrease in treatment satisfaction [-1.9(6.76)]. Better glycemic control in the combination group at study end accompanied improved treatment satisfaction. Furthermore, at trial end, perceived fear of hyperglycemia was significantly lower in RSG+SU group as compared to SU group [1.7(1.83) vs 3.1(1.99), $p<0.001$]. In addition, perceived fear of hyperglycemia decreased from baseline in RSG+SU group [-0.5(2.27)] and increased from baseline in SU group [+0.9(2.22)]. Superior glycemic control afforded by RSG+SU therapy was not associated with a significant difference in perceived fear of hypoglycemia compared to SU monotherapy at study end [0.2(1.73) vs 0.1(1.45), $p=0.66$].
Conclusion: In elderly T2D patients, improved glycemic control afforded by early addition of RSG to SU was associated with a significant increase in patient satisfaction with treatment as compared to progressive SU uptitration. Increased patient satisfaction could benefit long-term treatment adherence and glycemic control.

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Thiazolidinediones: water retention and other effects

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Predictors of rosiglitazone induced fluid retention

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Background and Aims: The clinical features that predict the occurrence of Rosiglitazone (RSG) associated fluid retention in type 2 diabetes mellitus T2DM) are currently unknown. In a cohort study of T2DM patients on background anti-diabetic medications with sulphonylurea or sulphonylurea plus metformin, we examined the effect of 12 weeks treatment with RSG 4 mg BD on changes in haematocrit (Htc). A reduction in Htc of 0.5% or more by the end of treatment was taken as an index of plasma volume expansion.
Materials and Methods: We studied 381 T2DM patients (M 67%, F 33%) mean age 60 ± 9 (SD) yrs, mean duration of diabetes 9.5 ± 6.2 (SD) yrs. A panel of 22 baseline demographic, clinical, biochemical and hormonal variables were measured and compared between those who showed volume expansion and those who did not. Further we analysed baseline variables in the whole cohort using the dependent variable, Htc as continuous variable. Both multiple logistic and linear regression analyses were carried out. Variables that were not linearly related to change in Htc were log transformed. Stepwise and backward model selection techniques, adjusting for baseline Htc, were utilised to find covariates with the strongest relationship to change in Htc.
Results: Complete data set was available in 344 patients, of these 260 (M 63%, 37% F) showed evidence of volume expansion, but 84 (80% M 20% F) did not. Htc reduction of ≥ 0.5% was accompanied by a significant fall in haemoglobin (Hb) and plasma albumin concentration and increase in total body water (TBW) and extracellular fluid (ECF) (by bioimpedance) (all $p<0.05$) confirming plasma volume expansion. Variables significantly related at the 5% level in univariate analysis with Htc (<0.5% vs. ≥ 0.5%) as the categorical dependent variable were: age, gender, systolic blood pressure (SBP), fasting insulin, serum creatinine, TBW and ECF. Using a logistic regression model with Htc as the categorical dependent variable (<0.5% vs. ≥ 0.5%) gave the following results, odds ratios with (95% confidence intervals) are presented. Female sex, 4.20 (2.13, 8.28) $p<0.001$, baseline Htc 1.16 (1.07, 1.25) $p<0.001$, SBP 1.02 (1.01, 1.04) $p=0.015$ and fasting insulin 0.995 (0.990, 0.999) $p=0.044$ emerged as baseline factors predicting fluid retention. When Htc was assessed as a continuous variable the predictors of Htc changes were: regression coefficients with (95% confidence intervals) female sex -1.84 (-2.41,-1.27) $p<0.001$, baseline Htc -0.21(-0.29, -0.14) $p<0.001$ and age -0.06 (-0.09, -0.03).
Conclusion: Whether Htc was treated as a binary or continuous variable female sex and baseline Htc emerged as key predictors of RSG induced volume expansion. Age was strongly related to continuous change in Htc whilst SBP and baseline insulin were related to Htc categorical change. These findings will prove useful in informing management strategies aimed at minimising RSG related fluid retention adverse effects.
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A comparison of the effects of pioglitazone versus rosiglitazone on edema and weight gain

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Background and Aims: Thiazolidinediones (TZDs) have been associated with the development of edema in 5–15% of patients and an average weight gain of 1–5 kg in treated patients. However, no direct comparison of the two TZDs, pioglitazone HCl (PIO) and rosiglitazone maleate (ROSI), on either of these measures has been reported. We, therefore, report the comparative effects of these two TZDs on edema and weight gain of patients participating in a prospective, randomized, double blind, trial with the primary objectives to compare the maximal doses of these agents on lipid and glycemic parameters.
Materials and Methods: Subjects with a diagnosis of type 2 diabetes with fasting triglyceride (TG) levels ≥150 mg/dL and <600 mg/dL, fasting LDL-C levels <130 mg/dL, and NYHA Class I and II were enrolled in this study from multiple sites. Previous therapy included either diet alone (25%) or oral monotherapy. After 4 weeks of placebo treatment, subjects were treated with PIO (n=400; 30 mg QD for 12 weeks followed by 45 mg QD for an additional 12 weeks) or ROSI therapy (n=402; 4 mg QD followed by 4 mg BID for the same intervals). Peripheral edema was clinically categorized as 0 (none) through 4 (deep pitting) at each visit.
Results: At baseline, the mean weight (±SE) was 93.7 ± 1.1 kg and 92.5 ± 1.1 kg for PIO and ROSI respectively. The mean maximum weight increase at endpoint was similar for PIO vs ROSI (3.0 ± 0.2 kg vs 2.7 ± 0.2 kg, p=0.157). The prevalence of edema for PIO vs ROSI was similar at baseline (18% vs 17%) and changes in edema from baseline to endpoint (LOCF) are as follows:

	No Edema in Edema	No Change Edema	Improving Edema	Worsening	p-value
PIO (n, %)	229 (62.7)	66 (18.1)	21 (5.8)	49 (13.4)	NS
ROSI (n,%)	233 (65.1)	54 (15.1)	25 (7.0)	46 (12.8)	

Only one episode of CHF was reported (occurring in a ROSI treated subject).
Conclusions: With monotherapy, weight gain and edema are similar for both TZDs. In addition, baseline rates of edema are high in this population.
Support: Eli Lilly and Company and Takeda Pharmaceuticals North America, Inc.

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Effects of pioglitazone on water volumes in patients with type 2 diabetes

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Background and Aims: The weight gain, increase in oedema and changes in haematocrit and haemoglobin that occasionally occur in patients treated with thiazolidinediones may indicate haemodilution, possibly due in part to an increase in intravascular or extravascular fluid. This 12-week, single-centre, randomised, placebo-controlled, double-blind study aimed to describe changes in water volumes in patients with type 2 diabetes treated with pioglitazone.
Materials and Methods: Males and post-menopausal females aged 40–75 years, inclusive, with type 2 diabetes (HbA_{1c} level of >6.5% and ≤10.0%), a BMI ≤35 kg/m^2 and either drug-naïve or receiving a stable dose of sulphonylurea or metformin (for ≥3 months prior to entry) were randomised to receive add-on therapy with either pioglitazone (15 mg/day titrated up to 45 mg/day during the first 3 weeks, depending upon tolerability; n=22) or placebo (n=20) for 12 weeks. The primary efficacy variables were total body water (TBW), extracellular water (ECW) and % change in plasma volume. HbA_{1c} and fasting plasma glucose (FPG) were also measured.
Results: Treatment with pioglitazone over 12 weeks resulted in a decrease in TBW by 1.7% and increases of 4.1% in ECW and 6.4% in plasma volume. Except for the ratio ECW/TBW, there were no statistically significant differences between the pioglitazone and placebo groups (see table). Mean HbA_{1c} decreased by 0.8% in the pioglitazone group and 0.1% in the placebo group. FPG decreased by 1.7 mmol/L in the pioglitazone group and 0.2 mmol/L in the placebo group. Pioglitazone was safe and well tolerated and all patients were titrated up to the maximum dose of 45 mg/day. Peripheral oedema and hypoglycaemia were each experienced in one patient.
Conclusion: This study shows that the changes in body water volumes with pioglitazone treatment were small and mainly represented an increase in interstitial water.

Parameter	Pioglitazone change from baseline (mean±SD)	Placebo change from baseline (mean±SD)	p-value
TBW (L)	-0.98 ± 4.45	-0.35 ± 1.74	0.753
ECW (L)	0.83 ± 1.51	0.31 ± 0.52	0.371
ECW/TBW (%)	2.35 ± 3.31	0.94 ± 1.83	0.031
Plasma volume (%)	6.42 ± 14.0	4.02 ± 10.3	0.544

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Increased body water content is the major cause of weight gain with pioglitazone despite improved hemodynamic parameters in type 2 diabetes

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Background and Aims: Pioglitazone (P), an insulin sensitizer and Glipizide (G), an insulin secretagogue are commonly used to treat type 2 diabetes. Use of P has been associated with substantial weight gain despite improvement in diabetic control. The effects of P vs. G on body water, body composition and hemodynamic parameters simultaneously have never been studied in diabetic humans.
Materials and Methods: To address these questions, we studied 21 subjects with type 2 diabetes randomly assigned to either 45 mg of P (n=10) or 10 mg of G (n=11). Body water content was measured with deuterated water, body composition by DXA and CT scans and cardiac output and systemic vascular resistance by acetylene techniques before and after 12 weeks therapy with either drug. The groups were matched for age, BMI, weight, body fat and HbA_1c concentrations.
Results: While P was associated with an increase ($p<0.001$) in body water (+2.4 ± 0.5 L), there were no changes (p=0.9) with G (+0.5 ± 2.0 L). This accounted for about 75% of the total weight gain (+3.1 ± 2.0 kg) observed with P. Therapy with G did not lead to any changes in body weight (+0.5 ± 0.5 kg). While there was a decline in total abdominal (-32.2 ± 19 cm^2) and visceral fat area (-16.1 ± 8 cm^2) with P, there were increases in both total abdominal (+38.4 ± 17 cm^2) and visceral fat area (+19.1 ± 9 cm^2) with G ($p<0.02$ for differences between groups). In contrast, total body fat content tended to increase with P (+0.9 ± 0.8 kg; p=0.15), whereas therapy with G did not lead to any changes (-0.2 ± 0.4 kg). While therapy with P tended to reduce (p=0.05) diastolic (-8.4 ± 4 mmHg) and mean (–9.5 ± 5 mmHg; p=0.08) blood pressures there were no changes with G of systolic (–1.4 ± 5 mmHg), diastolic (-0.3 ± 2 mmHg) or mean (–0.7 ± 3 mmHg) blood pressures. Concomitantly, there was a significant ($p<0.001$) reduction in systemic vascular resistance with P (2785 ± 336 vs. 2227 ± 136 dynes/sec/m^2) but not with G (2556 ± 205 vs. 2446 ± 223 dynes/sec/m^2). There were no changes in catecholamine concentrations with either therapy.
Conclusion: Our results show that 12 weeks of therapy with pioglitazone in type 2 diabetes led to a) significant increase in total body water that accounted for 75% of the total weight gain; b) redistribution of body fat content and c) reductions in blood pressure and systemic vascular resistance despite unchanged circulating catecholamine concentrations.
Support: Takeda Pharmaceuticals

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Effect of pioglitazone on microcirculation parameters in patients with type 2 diabetes treated with insulin

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Background and Aims: Mild to moderate peripheral oedema is a side effect that occurs more frequently with pioglitazone than with metformin or sulphonylureas. Mechanisms of oedema formation include precapillary vasodilatation, increased capillary wall permeability and attenuation of postural vasoconstriction. This single-centre, 8–10 week, randomised, placebo-controlled, double-blind study assessed microvascular function to identify the mechanisms underlying peripheral oedema formation with pioglitazone.
Materials and Methods: Subjects (aged 40–80 years) with type 2 diabetes (HbA_{1c} 6.5–10%) were randomised to pioglitazone (15 mg/day which was titrated to 30 mg/day, if required; n=14) or placebo (n=15). The primary endpoint was capillary filtration capacity (CFC). Secondary endpoints included isovolumetric venous pressure, capillary pressure, postural vasoconstriction, capillary recruitment and vascular endothelium growth factor (VEGF).
Results: Pioglitazone did not increase CFC (decrease of 0.15×10^{-3} mL/min/100 mL/mmHg versus an increase of 0.19×10^{-3} mL/min/100 mL/mmHg in the placebo group), decrease postural vasoconstriction (decrease of 21.2% for dorsum of the foot versus 22.5% for placebo) or significantly alter any of the other microcirculation variables. VEGF increased from baseline in the pioglitazone group (increase of 60.1 pg/mL), but this did not differ from placebo (10.6 pg/mL; p=0.463). The incidence of adverse events was similar between the two groups and one patient in each group experienced oedema; VEGF was decreased in the pioglitazone-treated patient.
Conclusion: In patients with type 2 diabetes on insulin, CFC and capillary pressure, the major factors influencing fluid extravasations, are not ubiquitously increased by pioglitazone. The lack of an increase in VEGF with pioglitazone suggests that VEGF-mediated increases in transvascular fluid movement are unlikely in this group of patients. Furthermore, pioglitazone does not impair postural vasoconstriction, an oedema-preventing mechanism, in contrast to previous findings with calcium antagonists

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Irreversible TZD-induced pubic fat hypertrophy: an embarrassing side-effect

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Background and Aims: Thiazolidine-diones (TZDs), a relatively new class of oral glucose lowering drugs, are selective ligands of the nuclear transcription factor peroxisome-proliferator-activated receptor (PPAR-γ), which is mainly located in fat tissue. TZDs have a beneficial effect on insulin resistance. Side-effects are fluid retention and weight gain mainly caused by an increase in subcutaneous fat mass. Here we describe two patients who developed bulky, selective, hypertrophy of the pubic fat area causing mechanical discomfort and severe cosmetic embarrassment.
Case Presentations: Patient A, a 54-year-old woman, had hypertension, hypertriglyceridemia, increased liver fat and type 2 diabetes for 9 years. She was obese (BMI: 33.6 kg/m2) with pronounced truncal obesity, and a hint towards a buffalo hump and moonface. Cushing's disease was excluded and screening for mutations in genes associated with lipodystrophy (LMNA, PPARγ, Emerin, AGPAT2 and BSCL2; courtesy dr. R. Hegele) was negative. Her diabetes was controlled by CSII with a daily insuline dose of ~290 Units. To improve insulin sensitivity and reduce insulin requirements, pioglitazone 30 mg qd was added, with moderate effect. After 9 months she reported a gradual increasing pubic mass, at PE consisting of fat. After discontinuation of pioglitazone the consistency changed, but the size was unaltered and eventually she underwent plastic surgery reconstruction with removal of a fat mass of ~2 kg.
Patient B, a 53-year-old man, with a history of hypertension, gout and obesity (BMI: 36 kg/m^2), had type 2 diabetes diagnosed and treated with the combination of rosiglitazone 8 mg qd and metformin 500 mg bid. Over the next 4 months, his weight increased by 9 kg and he reported pronounced peri-orbital swelling and an enlarging pubic mass. He had diplopia due to exophthalmus, caused by retrobulbar lipomas. At PE, also the pubic mass consisted of fat tissue. After discontinuation of rosiglitazone, the fat masses did not regress. He was treated by retroorbital dissection of the lipomas. There were no mutations in the genes of LMNA, PPARγ, AGPAT2 and BSCL2.
Results: Fat tissue morphology of the pubic fat (patient A) and of the retroorbital lipomas (patient B) showed adipocytes being rather heterogeneous in size with prominent inflammatory cells. Molecular studies of tissue samples are ongoing.
Conclusion: These two cases strongly suggest that both pioglitazone and rosiglitazone can induce irreversible fat hypertrophy in specific locations, such as the pubic area and the peri-orbital space. Whether these side-effects can be attributed to specific abnormalities in fat biology in these patients remains to be determined.

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Rosiglitazone added to metformin reduces urinary albumin/creatinine ratio and ambulatory blood pressure in subjects with microalbuminuria and type 2 diabetes

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Background and Aims: Microalbuminuria (MA) is an independent risk marker for cardiovascular disease and renal progression in type 2 diabetes mellitus (T2DM). Reduction in albumin excretion may be indicative of improvement in vascular status. This prospective, randomised, double blind, active controlled study assessed the long-term glycaemia-independent efficacy of rosiglitazone (RSG) in reducing urinary albumin/creatinine ratio (UACR) in T2DM subjects with MA.
Materials and Methods: Following at least 4 weeks of background metformin (MET; ≥ 1g/day) 389 subjects were randomised to the addition of RSG (4 mg once daily) or glibenclamide (GLB, 5 mg once daily) for 8 months. Both RSG and GLB could be titrated to achieve a fasting blood glucose target of ≤ 6.7 mmol/l to ensure comparable glycaemic control. The primary endpoint was change from baseline in UACR within the RSG+MET group. Mean 24-hour ambulatory blood pressure (ABP) was a secondary endpoint.
Results: As intended, both the baseline and changes in glycaemic parameters were comparable between the two groups.

UACR (µg/mg)	**RSG + MET (n = 192)**	**GLB + MET (n = 180)**
Baseline (geometric mean)	65.7	63.5
% change from baseline (95% CI)	–22.8 (–32.3, –11.9)**	–7.1 (–19.6, 7.4)
Comparison with GLB + MET	–15.5 (-29.6, 1.5)‡	
24-hour systolic ABP (mmHg)	**n = 94**	**n = 87**
Baseline (mean±SD)	135.9 ± 12.2	138.3 ± 12.7
Change from baseline	–1.30 (–3.2, 0.6)	1.28 (–0.9, 3.5)
Comparison with GLB + MET	–3.4 (–6.0, –0.7)*	
24-hour diastolic ABP (mmHg)	**n = 94**	**n = 87**
Baseline (mean±SD)	79.7 ± 7.5	78.5 ± 7.8
Change from baseline	–2.30 (–3.4, –1.2)**	0.3 (–1.0, 1.6)
Comparison with GLB + MET	–2.5 (–4.2, –0.9)*	

Intent to treat (ITT) subjects with last observation carried forward (LOCF) *$P \leq 0.01$, **$P \leq 0.001$, ‡$P = 0.071$

In the ITT without LOCF population, the difference in change from baseline in UACR between RSG + MET and GLB + MET was statistically significant (–19.5%, $P < 0.05$).
Conclusion: Long-term treatment with RSG has potentially beneficial effects on UACR in T2DM subjects with MA independent of glycaemia, perhaps due to salutary effects on ABP.

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Ten years of rosiglitazone clinical trial experience: a hepatic monitoring update

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Background and Aims: A signal of hepatoxicity was observed in clinical trials (CT) with troglitazone (TRG), the first generation thiazolidinedione (TZD); the incidence of alanine aminotransferase (ALT) > 3x the upper limit of the laboratory reference (ULRR) in CT was 1.9% (n = 48/2510) vs. 0.6% (n = 3/475) with placebo (PBO) control. Subsequent post-marketing reports of acute liver failure prompted withdrawal of TRG from the market. This prompted Lebovitz *et al.* to examine the CT liver enzyme results for rosiglitazone (RSG), the second marketed TZD. RSG was evaluated in 22 double-blind comparator and open-label clinical studies with 5,006 patients treated with RSG as monotherapy or combination therapy for 5,508 person-years (PY). The data presented here update this report with hepatic monitoring from ten years of CT experience with RSG.
Materials and Methods: Liver enzyme levels were assessed at screening, baseline, and every 4 weeks for the first 3 months of treatment and at 6- to 12-week intervals, thereafter. Patients were allowed to participate if they had total bilirubin, alkaline phosphatase, ALT, or aspartate aminotransferase (AST) levels ≤ 2.5x the ULRR. ALT values > 3x the ULRR were considered to be of potential clinical concern.
Results: Through 2004, exposure to RSG has increased to 8,864 PY in 38 clinical studies representing a 61% increase since the earlier analysis. There continues to be no excess reporting of liver enzyme elevation following the substantial increase in CT experience. The rate per 100 PYs remains low and comparable with observations for PBO and comparator agents. Of note, liver function test monitoring for second generation TZDs has been relaxed to evaluation at the initiation of therapy and as clinically indicated.

	All RSG N = 7,714, PY = 8,864			**PBO** N = 680, PY = 188			**SU + MET + Insulin** N = 2,901, PY = 1,668		
> 3xULR	N	n (%)	rPY	N	n (%)	rPY	N	n (%)	rPY
ALT	7,429	23 (0.3)	0.3	639	1 (0.2)	0.5	2,792	8 (0.3)	0.5
AST	7,429	17 (0.2)	0.2	639	1 (0.2)	0.5	2,793	5 (0.2)	0.3
Alkaline phos.	7,390	5 (0)	0	639	0	0	2,750	3 (0.1)	0.2
Total bilirubin*									
> 1,5xULR	7,335	37 (0.5)	0.4	639	5 (0.8)	2.7	2,741	23 (0.8)	1.4

rPY = Rate/100 patient years of exposure; SU = sulphonylurea;
MET = metformin
> 1,5xULR

Conclusion: Extensive hepatic monitoring across ten years of CT experience is consistent with earlier studies in demonstrating that RSG is not associated with hepatotoxic effects.
Conducted by GlaxoSmithKline

PS 65

Oral antidiabetic agents: comparative studies

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Assessment of insulin secretion in response to a mixed meal in type 2 diabetic patients. Effects of short-term repaglinide and glibenclamide administration

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Background and Aims: To compare the acute effect of repaglinide and glibenclamide on physiological dynamic features of insulin secretion in response to a mixed meal in type 2 diabetes.
Material and Methods: To avoid the confounding effect of chronic plasma glucose reduction, 7 naïve treated type 2 diabetic patients (4M/3F; 61 ± 2 years old; BMI 27.5 ± 1.2 kg/m^2; HbA_1c 7.3 ± 0.2%; mean±se) were studied after 2 day administration of placebo (PL), glibenclamide (GLIB: 5 mg b.i.d.), and repaglinide (REP: 1 mg b.i.d) with the last dose assumed 30 min before the ingestion of a standard mixed meal (600 Kcal; 50% CHO, 32% lipids, and 18% protein). Studies were performed 1–2 weeks apart in a random order. Plasma glucose, C-peptide and insulin concentrations were measured in the basal state and for 300 min after meal ingestion. Dynamic features of insulin secretion were determined according to the mathematical model proposed by Mari et al (Diabetes 51, Suppl. 1:S221, 2002).
Results: Fasting plasma glucose was comparable in all conditions (PL: 8.1 ± 0.7; GLIB: 6.7 ± 0.4; REP: 7.3 ± 0.6 mmol/l). There was no significant difference in the 0–60 min rate of plasma glucose increment (0.07 ± 0.01, 0.04 ± 0.01, and 0.06 ± 0.01 mmol/l/min, respectively), while incremental AUCs were lower with GLIB and REP (2.5 ± 0.4 and 2.3 ± 0.5 mmol/l·min) as compared to placebo (3.7 ± 0.7; both $p<0.05$). Maximal plasma glucose increments also were lower with drugs (GLIB: 5.4 ± 0.7, REP: 4.5 ± 0.6 mmol/l) as compared to PL (6.4 ± 0.7; both $p<0.05$). Drug administration was associated with an upward shift in the beta-cell dose response with significant increase of insulin secretion at 7.5 mmol/l glucose (GLIB: 183 ± 20, REP: 168 ± 34 pmol/min/m^2) as compared to PL (116 ± 30; both $p<0.05$). The slope of the dose response did not differ (PL: 36 ± 15; GLIB: 38 ± 10; REP: 52 ± 12 pmol/min/m^2/mM; p=NS). Potentiation factor was flat for PL, increased early with REP, and was sustained over the time with GLIB. Rate sensitivity, which expresses early insulin release, tended to be greatest with REP (646 ± 160 pmol/m^2/mmol), intermediate with GLIB (502 ± 131), and lowest with PL (405 ± 152). Finally, in multivariate linear regression in the whole group, the mean relative postprandial glucose increment was significantly correlated to both insulin secretion at 7.5 mmol/l glucose and rate sensitivity ($r2=0.47$, $p<0.005$).
Conclusion: Under the condition of acute administration and in the absence of overall changes in glucose control, 2 day administration of 1 mg repaglinide is at least as effective as glibenclamide in improving glucose tolerance after the ingestion of a mixed meal. This effect appears to be related to beta-cell function improvement, which has different characteristics with repaglinide and glibenclamide.

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Improvement of beta cell function by nateglinide and repaglinide in type 2 diabetic patients: a randomized controlled double blinded and double dummy multicentre study

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Background and Aims: Insulin resistance and reduced β-cell insulin secretary capacity are important factors in the pathogenesis of type 2 diabetes. Repaglinide and nateglinide are short-acting insulin secretagogues, which promote mealtime insulin release. To evaluate the effect on glycemic control, β-Cell function and insulin resistance of nateglinide versus repaglinide in type 2 diabetic patients, a randomized controlled double-blinded and double-dummy multicenter clinical trial was conducted.
Materials and Methods: 230 Chinese type 2 diabetic patients were enrolled from 5 clinical centers. The patients were divided randomly into repaglinide group (repaglinide 1.0 mg,tid, n=115) or nateglinide group (nateglinide 90.0 mg tid, n=115)treatment group. The trial consisted of a 4 week equilibrated period followed by 12 week treatment period.
Results: 223 patients (96.9%) finished the trail. At baseline and end of the clinical trial, standard mixed meal tolerance tests were measured. After treatment, fasting blood glucose (FBG) decreased 1.68 mmol/L (17.27%) and 1.17 mmol/L (12.53%) respectively in (repaglinide and nateglinide group, with the means of HbA_1c were lowered 1.21% and 0.68% respectively. And the reductions in FBG and HbA_1c were significantly different in two groups. The postprandial glucose peaks declined and areas under the curve (AUC) of blood glucose 0~120 min were remarkably reduced in both groups ($P<0.0001$). AUC of insulin were much enlarged than baseline in repaglinide and nateglinide group ($P\leq0.0001$). Furthermore, rise rates of insulin concentrations over the first 30 min increased significantly in both groups ($P<0.0001$), which suggested both nateglinide and repaglinide promoted early phase insulin secretion. HOMA-IR and β-cell function indexes quantified as ($\Delta I_{30}/\Delta G_{30}$) or β-cell function adjusted indexes quantified as ($\Delta I_{30}/\Delta G_{30}$/HOMA-IR) was improved significantly in repaglinide and nateglinide group.
Conclusion: Repaglinide and nateglinide restore early phase insulin secretion during the postprandial period, which could reduce mealtime glucose excursions, FBG, as well as HbA_1c, and could be of advantage to β-cell function and insulin resistance.

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Evaluation of the efficacy of repaglinide compared with glibenclamide in newly diagnosed type 2 diabetic patients by continuous glucose monitoring system

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Background and Aims: To compare the efficacy of repaglinide (Repag) with glibenclamide(Glib) on daily blood glucose profile, especially on postprandial blood glucose profile, in newly diagnosed type 2 diabetic(T2DM) patients by continuous glucose monitoring system(CGMS).
Materials and Methods: Twenty newly diagnosed T2DM patients by 75g oral glucose tolerance test (75gOGTT) were randomly assigned into Repag group (6 males and 4 females, 45 ± 5 years old) (0.5 mg tid) or Glib group (5 males and 5 females, 47 ± 4 years old) (2.5 mg bid), and they were all treated for 4 weeks. To evaluate the daily change of blood glucose, glucose monitoring was carried out with CGMS before and after treatment. The meal menu and mealtime were asked to basically maintain the same during the first and second CGMS examination.
Results: (1) All the patients were well in compliance with CGMS examination. The average time of CGMS examination was 69.6 ± 3.4 hours, and an average of 836 ± 41 blood glucose values were recorded. (2) The average blood glucose, standard deviation of blood glucose were all decreased in both groups ($P<0.05$, respectively). The blood glucose values in various times of the overall day were decreased ($P<0.05$,respectively) in Repag group,and the blood glucose in most time of the overall day except postlunch were also decreased ($P<0.05$,respectively)in Glib group.(3) The decrease of average blood glucose levels (Repag:11.5 ± 1.4 to 8.3 ± 0.7 vs Glip:11.0 ± 1.0 to 9.2 ± 0.8 mmol/L),standard deviation of blood glucose levels(Repag:1.8 ± 0.3 to 1.2 ± 0.3 vs Glip:1.8 ± 0.2 to 1.7 ± 0.3 mmol/L), 2 h postprandial blood glucose levels (Repag:15.6 ± 3.3 to 10.9 ± 1.4 vs Glip:15.5 ± 2.3 to 12.9 ± 1.9 mmol/L) and peak postprandial blood glucose levels in Repag group were decreased more than that in Glib group, while the blood glucose at 3:00am decreased less (Repag: 8.4 ± 0.9 to 7.5 ± 0.5 vs Glip: 8.2 ± 0.6 to 6.4 ± 0.3 mmol/L) ($P<0.05$). The pre-prandial and bedtime blood glucose in both groups were comparable ($P>0.05$).
Conclusion: It is confirmed with CGMS technique, that Repag therapy could significantly decrease postprandial hyperglycemia and make the daily blood glucose excursion smoother compared with Glib in newly diagnosed T2DM patients. So it can be concluded that repaglinide is more appropriate for actual life in newly diagnosed T2DM therapy and CGMS is a very helpful tool in diabetes care.

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Cardiac effects of sulfonylureas: comparison between glibenclamide and gliclazide in mouse ventricular myocytes

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Background and Aims: 35 years after the UGDP study (University Group Diabetes Program), the cardiac effects of sulfonylureas are still a matter for debate. Are sulfonylureas deleterious by suppressing ischemic preconditioning or protective by decreasing arrhythmia during acute ischemia? These drugs target sulfonylurea receptor (SUR), the regulatory subunit of K_{ATP} channels present in neuronal, cardiac and muscular cells. In the heart, these channels are activated during transient periods of ischemia and hypoxia and they contribute to action potential shortening which results in reduced myocardial contractility and oxygen consumption. Here we investigated the impact of sulfonylurea treatment in type 2 diabetics on cardiac cell function under ischemic conditions. We used mouse ventricular myocytes and compared the effects of two sulfonylureas displaying different selectivity for K_{ATP} channels, gliclazide and glibenclamide. Gliclazide is specific for pancreatic K_{ATP} channel with a very low affinity for the myocardial SUR2A receptor, while glibenclamide interacts in the same range of affinity with both pancreatic SUR1 and cardiac isoforms. We studied the effects of plasmatic therapeutic concentrations of these two sulfonylureas on action potentials (AP) and calcium transients under metabolic inhibition (MI).
Materials and Methods: Single ventricular myocytes were isolated from C57/black6 mice using standard enzymatic methods with collagenase. MI was caused by the addition of sodium cyanide and iodoacetic acid in a substrate-free Tyrode solution and investigated in three different conditions including control (MI), MI + 50 µM gliclazide and MI + 0.5 µM glibenclamide. AP were recorded using the whole-cell patch-clamp method. Calcium transients were elicited by field stimulation of fluo-3 loaded cells with two Pt electrodes at 1Hz and recorded using confocal microscopy.
Results: MI caused a hyperpolarization of ventricular myocytes which was accompanied by a progressive shortening of action potential duration. In the presence of gliclazide, MI caused similar effects. In contrast, in the presence of glibenclamide, hyperpolarization was preceded by a depolarization ranging from 10 to 40 mV. The progressive shortening of action potential occurred but was followed by a systematic rigor contraction. In parallel, MI suppressed calcium transient. The block developed progressively and was achieved after 231.5 ± 12.2 s (n=10). A complete recovery was observed upon wash out. In the presence of gliclazide, no difference was observed in the time course of block (228.4 ± 14.5 s, n=11) and recovery was obtained for 91% of cells. In the presence of glibenclamide, inhibition of calcium transient was faster (173.7 ± 14.1 s, n=9) with less recovery upon wash out (40%) and increased rigor contraction.
Conclusion: Gliclazide, at therapeutic concentration, has no effect on electrical activity and calcium transient of cardiac ventricular myocytes subjected to MI. In contrast, glibenclamide worsens the effects of MI, leading to cell death. Therefore, glibenclamide but not gliclazide might have deleterious effects on ischemic myocardium. One question is raised: are all sulfonylureas equally beneficial for diabetic patients facing myocardial ischemia?

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Effect of metformin vs repaglinide on postprandial glycaemia, plasma lipoproteins and free fatty acids in non-obese patients with type-2 diabetes (T2DM)

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Background and Aims: Postprandial carbohydrate and lipid metabolism have been linked to the development of macrovascular complications in diabetes. Little is known about the effect of oral antiglycaemic drugs on postprandial metabolic variables in patients with T2DM, and previous studies included obese patients with T2DM. Metformin (Met) improves insulin action and is supposed to possess cardioprotective potentials in obese patients with T2DM. Repaglinide (Rep) is a short-acting insulin-secretagogue targeting postprandial plasma-glucose excursions. Rep has similar or superior antiglycaemic effect compared to Met in obese and non-obese patients with T2DM. The aim of the study was to compare the effect of Met versus Rep on postprandial metabolic variables in non-obese patients with T2DM.
Materials and Methods: Single-center, double-blind, randomised, double-dummy, cross-over-study of 96 non-obese (BMI ≤ 27 kg/m^2) Caucasian T2DM-patients, age ≥ 40 years, treated with either diet-only or oral hypoglycemic agents having HbA_{1c} between 6.5% and 9.5%. After a 1 month run-in on diet-only treatment patients were randomised to either Rep 2 mg × 3 followed by Met 1g × 2 or vice versa each for a period of 4 months with a 1 month wash-out between interventions. Fasting and postprandial metabolism were evaluated by a meal-test before and after each treatment-period. After an overnight fast patients were served a standard fatty meal of 3.515 kJ containing 54 energy % fat, 13% protein and 33% carbohydrates. Patients took the morning doses of the study-medications immediately prior to the meal. Only drinking water was allowed postprandially. Blood samples were drawn at the time points 0 (fasting), 11/2, 3, 41/2 and 6 hours (postprandially) and analysed for plasma-glucose, serum-insulin, serum-C-peptide, plasma-lipids and serum free fatty acids. Postprandial profiles of metabolic variables were compared by area under the curve (AUC) for all time-points during 0–6 hours after the meal.
Results: Fasting values and AUC for serum-insulin and serum-C-peptide were significantly lower during Met vs Rep (Mean [95%CI] AUC-difference Met vs Rep: Insulin −12505 minutes x pmol/l [−16603; −8407]; C-peptide −122988 minutes x pmol/l [−152719; −93257]; p< 0.001). Fasting values and AUC for circulating levels of glucose, total cholesterol, HDL-cholesterol, triglyceride and free-fatty acids were not significantly different between treatments. HbA_{1c} was higher during Met vs Rep (Mean [95%CI] HbA_{1c}-difference Mep vs Rep 0.3% [0.02; 0.55], p=0.032).
Conclusion: Metformin resulted in lower fasting and postprandial serum insulin-levels, but a higher level of HbA_{1c} as compared with Repaglinide in non-obese patients with T2DM. Otherwise the two drugs had similar effects on postprandial carbohydrate and lipid regulation.
Support: Novo Nordisk A/S

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56-week effects of pioglitazone vs glyburide on glycemic control, lipids, and LDL fractionation in subjects with recently diagnosed type 2 diabetes

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Background and Aims: Long-term glycemic control, lipid levels (triglycerides [TG], total cholesterol, HDL-C, and LDL-C), and LDL fractionation were compared between pioglitazone (PIO) and glyburide (GLY) in subjects with recently diagnosed type 2 diabetes and previous unsuccessful treatment with diet and exercise. The primary end point was HbA_1c.
Materials and Methods: In this multicenter, double-blind, double-dummy, randomized active-comparator study, 502 treatment-naïve type 2 diabetes subjects were enrolled (aged 18–80 years, diagnosis <2 years prior). After 2 weeks of screening, subjects were randomly assigned to once daily PIO 15 mg or GLY 5 mg and entered a 16-week titration period. At 4-week intervals thereafter, the initial dose was increased up to 45 mg daily (in 15-mg increments) for PIO or 15 mg daily (in 5-mg increments) for GLY; glucose control was optimized to achieve fasting plasma glucose (FPG) levels >69 and <141 mg/dL. Thereafter, subjects continued their final titration regimen in a 40-week treatment period.

Results: Least Squares Mean (SE) Changes From Baseline

		Baseline	Week 56 Change
A1c (%)	PIO	9.14 (0.087)	−2.07 (0.086)
	GLY	9.20 (0.084)	−2.02 (0.083)
TG (mg/dL)	PIO	204.2 (9.90)	−37.2 (6.51)*
	GLY	215.2 (9.63)	−15.6 (6.35)
HDL-C (mg/dL)	PIO	38.7 (0.67)	+6.6 (0.53)**
	GLY	37.4 (0.65)	+3.1 (0.51)
LDL-C (mg/dL)	PIO	122.6 (2.74)	+3.8 (2.00)
	GLY	126.6 (2.66)	−0.3 (1.96)
Total cholesterol (mg/dL)	PIO	199.6 (3.14)	+4.0 (2.38)
	GLY	204.5 (3.06)	−2.0 (2.32)
Average particle size (Å)	PIO	257.6 (0.40)	+3.8 (0.34)**
	GLY	257.1 (0.39)	+0.5 (0.33)
LDL large [Pattern A] (%) (a)	PIO	36.3 (1.45)	+14.3 (1.28)**
	GLY	34.2 (1.41)	+1.8 (1.24)
LDL small [Pattern B] (%) (a)	PIO	43.2 (1.60)	−13.3 (1.26)**
	GLY	45.9 (1.56)	−2.2 (1.23)

Baseline, n=251 per group.
PIO vs GLY: *P<0.05, **P<0.001. (a) Gradient gel electrophoresis

At week 56, PIO and GLY produced comparable reductions in HbA_1c. In the PIO group, TG levels were significantly reduced (P<0.05) and HDL-C levels were significantly increased (P<0.001) when compared to GLY. Significant increases in average LDL particle sizes, and overall shifts from small par-

ticles (Pattern B) to large particles (Pattern A) occurred with PIO (P<0.001) compared to GLY, without significant differences in LDL-C or total cholesterol levels. Significantly fewer subjects in the PIO group withdrew before week 56 because of lack of therapeutic efficacy or an adverse event (GLY: 44 [20.8%]; PIO: 25 [12.8%]; P=0.032).
Conclusion: In subjects with recently diagnosed type 2 diabetes and previous unsuccessful treatment with diet and exercise, PIO resulted in long-term (56-week) improvements in plasma lipids compared to GLY, including a significant increase in HDL-C levels and a significant decrease in TG levels, as well as overall improvements in LDL composition changes without significant differences in LDL-C or total cholesterol levels. These improvements with PIO occurred with no significant difference in glycemic control (HbA1c) compared to GLY at week 56.

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Impact of rosiglitazone given in addition to glimepiride treatment on cardiovascular risk markers, insulin resistance and β-cell dysfunction
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Background and Aims: This investigation was performed to assess the specific impact of rosiglitazone on cardiovascular risk markers, insulin resistance, β-cell function and adiponectin secretion in combination with sulfonylurea therapy.
Materials and Methods: One hundred and two patients from a double-blind, three-arm comparator trial (glimepiride+placebo (Glim+Plac): n = 30), glimepiride+4 mg rosiglitazone (Glim+4RSG): n = 31), or glimepiride+8 mg rosiglitazone (Glim+8RSG): n = 41); 46 women, 56 men, age (mean± SD) 62.8 ± 9.1 years, BMI 28.7 ± 4.5 kg/m^2, diabetes duration 6.4 ± 4.8 years, HbA_{1c} 8.1 ± 1.5%) were analyzed after 0 and 16 weeks of treatment. Observation parameters were: HbA_1c, glucose, $HOMA_{IR}$ score, insulin, intact proinsulin, and adiponectin. Insulin resistance was defined by elevated intact proinsulin values (< 11 pmol/l) or $HOMA_{IR}$ > 2.
Results: All parameters were comparable in the three groups at baseline. No changes were seen for any of the observation parameters with glimepiride alone. Significant dose-dependent improvements were observed after addition of rosiglitazone for fasting glucose (Glim+Plac: -9 ± 48 mg/dl/Glim+4RSG: -38 ± 47 mg/dl/Glim+8RSG: -46 ± 53 mg/dl), HbA1c (–0.1 ± 0.7%/-1.1 ± 1.2%/-1.3 ± 1.2%), insulin (+1.4 ± 6.2 µU/ml/-1.2 ± 5.3 µU/ml/ –3.7 ± 9.9 µU/ml), intact proinsulin (+1.6 ± 7.1 pmol/l/-2.0 ± 4.6 pmol/l/ –3.1 ± 6.1 pmol/l) and hsCRP (–0.21 ± 3.22 mg/dl/–1.69 ± 3.50 mg/dl/ -2.08 ± 3.49 mg/dl). After body weight adjustment, a significant additional contribution of PPARγ activation (p<0.001) was also detected for an increase in adiponectin (-0.5 ± 5.8 mg/l/+8.8 ± 22.9 mg/l/+14.3 ± 19.9 mg/l). The number of insulin resistant patients decreased only in the rosiglitazone treatment groups (Glim+Plac: from 53% to 60%, Glim+4RSG: from 52% to 29%, Glim+8RSG: from 54% to 22%).
Conclusion: Rosiglitazone provided an additional beneficial effect on blood glucose control, insulin resistance, β-cell function and cardiovascular risk markers when given in addition to glimepiride. Our results support the clinical rationale of combining rosiglitazone with sulfonylurea drugs in patients with type 2 diabetes.
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A comparison of nontraditional atherogenic markers with pioglitazone and rosiglitazone in patients with type 2 diabetes and dyslipidemia
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Background and Aims: The majority of patients with type 2 diabetes (T2D) demonstrate some degree of insulin resistance and associated metabolic abnormalities, such as dyslipidemia and increased expression of inflammatory markers, which increase the risk for cardiovascular disease. This prospective study evaluated the lipid-altering potential of pioglitazone (PIO) vs rosiglitazone (ROSI) in patients with T2D and dyslipidemia. A comparison of PIO and ROSI with respect to the effects on high-sensitivity C-reactive protein (hsCRP), plasminogen activator inhibitor 1 (PAI-1), low-density lipoprotein (LDL) particle concentration, and apolipoprotein (Apo) B are reported here.
Materials and Methods: This randomized, double-blind, multicenter, parallel group comparison of PIO and ROSI enrolled patients with T2D (WHO criteria) and dyslipidemia (fasting triglycerides [TGs] ≥150 and ≤600 mg/dL; fasting low-density lipoprotein cholesterol [LDL-C] ≤130 mg/dL). Previous therapies included either diet alone or oral antihyperglycemic agent monotherapy, excluding PIO or ROSI. All prior antihyperglycemic medications were discontinued at randomization. Patients were randomly assigned to either PIO (*n*=400) or ROSI (*n*=402) and received placebo during a 4-week washout. The placebo washout was followed by 24 weeks of PIO or ROSI monotherapy. Active PIO treatment started at 30 mg QD increasing to 45 mg QD at 12 weeks; ROSI treatment was initiated at 4 mg QD increasing to 4 mg BID at 12 weeks. Patients received no other lipid-lowering therapies before or during the study.
Results: There were no significant differences in baseline values between the PIO and ROSI groups. While PIO and ROSI produced similar changes in HbA1c (PIO, -0.7%; ROSI, -0.6%, *p*=0.129, between groups), TG levels significantly decreased from baseline with PIO (-12.0%) but increased from baseline with ROSI (14.9%; *p*<0.001, between groups).

Least Squares Mean (SE) Change From Baseline to End Point

Parameter	PIO	ROSI	*p* Value Between Groups
hsCRP (mg/L)			
n	348	333	
Baseline	7.0 (0.55)	6.6 (0.42)	–
Change at End point	–2.0 (0.33)*	–2.5 (0.33)*	0.288
PAI-1 (ng/mL)			
n	325	322	
Baseline	62.8 (2.34)	60.0 (2.28)	–
Change at End point	–10.4 (2.04)*	–11.7 (2.04)*	0.623
LDL-C (mg/dL)			
n	364	356	
Baseline	107.1 (1.33)	109.1 (1.36)	–
Change at End point	12.3 (1.60)*	21.3 (1.62)*	<0.001
LDL particle concentration (nmol/L)			
n	333	325	
Baseline	1,393.8 (19.77)	1,368.2 (20.64)	–
Change at End point	–50.5 (21.26)	110.5 (21.48)*	<0.001
LDL particle size (nm)			
n	333	325	
Baseline	20.0 (0.77)	20.1 (0.04)	–
Change at End point	0.5 (0.04)*	0.33 (0.04)*	0.005
Apo B (mg/dL)			
n	346	334	
Baseline	104.8 (1.06)	104.1 (1.05)	–
Change at End point	–0.2 (1.18)	10.6 (1.19)*	<0.001

*$p<0.001$ vs baseline based on mean change.
$p<0.05$ vs baseline based on mean change.

Conclusion: Overall, PIO and ROSI produced similar improvements in glycemic control and comparable decreases in measures of prothrombotic effects and inflammation. However, PIO significantly improved nontraditional biomarkers of cardiovascular risk, including TGs, LDL-C, LDL particle concentration and size, and Apo B, compared to ROSI.

PS 66
DPP-IV inhibitors and exenatide

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Effects of the short-acting dipeptidyl peptidase IV inhibitor PSN9301 and metformin alone and in combination on glucose tolerance and body weight in the *fa/fa* Zucker rat, and in a polygenetic rat model of diabetes

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Background and Aims: PSN9301 is a novel oral Dipeptidyl Peptidase IV (DP-IV) inhibitor that is unique in having a relatively rapid onset and short duration of action. It is currently in Phase II clinical development where its efficacy and safety are being evaluated in type 2 diabetic patients. The aim was to assess the acute and subchronic anti-diabetic efficacy of PSN9301 in both mono- and poly-genetic animal models in comparison to, and in combination with, metformin.
Materials and Methods: 13 week old male diabetic *fa/fa* Zucker rats were treated orally b.i.d. for 3 weeks with either vehicle (distilled water), PSN9301 60 mg/kg (group A), metformin at low 125 mg/kg (B) or high 300 mg/kg (C) dose, or with a combination of PSN9301 and these metformin doses (D, E). In a second study, 11 week old glucose-intolerant diet-induced obese (DIO) rats (fed a high fat diet from 4 weeks old) were treated orally b.i.d. for 2 weeks with either vehicle, PSN9301 60 mg/kg (A1) or metformin 125 mg/kg (B1), or a combination (D1). Various parameters were measured in one or both studies at various stages before and after treatment, including oral glucose tolerance (OGTT), 24 h glucose and insulin profiles, HbA_1c, and body weight gain.
Results: In the Zucker rats, treatments C, D and E improved glucose tolerance acutely (significant reductions in glucose area under curve; G-AUC) when administered at -5 min before OGTT, without distinct different effects on insulin release at day -1. After 2 and 3 weeks of treatment, all groups showed repeatedly improved tolerance curves and reduced G-AUC ($p<0.05$ vs vehicle), and most so in C, D and E. First phase insulin secretion was restored in A and D and means of 24 h blood glucose and insulin were also decreased, most effectively after combined treatment (D, E). HbA1c was slightly reduced in A and significantly in all other groups at day 18 ($p<0.05$). Body weight gain was more reduced in groups C, D and E than A. In the DIO study at day -7 no significant effects on G-AUC under an OGTT were observed on acute dosing in groups A1 and B1 whereas group D1 gave a significant reduction. In contrast, after 14 days treatment all groups gave significant reductions in the OGTT G-AUC, with slightly larger reductions evident in groups A1 and D1. Body weight gain was reduced after 14 days in all treatment groups with the reduction magnitude being group D1>B1>A1. Despite the reductions in body weight gain noted, no dramatic differences in food intake were noted between the groups over the courses of these studies.
Conclusion: Subchronic administration of PSN9301 restored early insulin secretion in male diabetic *fa/fa* Zucker rats, and when given alone in this and in the DIO rat model, its overall efficacy in improving blood glucose handling was similar to metformin at the doses used. Moreover, the combination of PSN9301 with metformin in these two different subchronic models of diabetes gave marked improvements in glucose tolerance and also in reductions in body weight gain, indicating that such a combination could prove to be an effective therapeutic approach especially in obese type 2 diabetics.

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Dipeptidyl-peptidase IV inhibition does not affect tissue permeability and extravasation of macromolecules in the fructose-fed insulin-resistant rat model

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Background and Aims: Reduced extravasation of macromolecules in skeletal muscle has been documented in the fructose-fed rat model, corroborating the hypothesis that a functional obliteration of muscle regional microcirculation might restrict access of nutrients and hormones to their target cells. Given the fact that dipeptidyl-peptidase IV (DPP-IV) cleaves bradykinin and substance P *in vitro*, the goal of this study was to assess the impact of a treatment with a DPP-IV inhibitor on the capillary permeability to albumin in various organs.
Materials and Methods: For these experiments, fructose-fed Sprague-Dawley rats were gavaged with GW825964X ($10\,mg \cdot kg^{-1} \cdot d^{-1}$; n=18) or the vehicle only (n=18) for 4 weeks before assessing the extravasation of albumin-bound Evans Blue (EB) dye *in vivo*. Unanaesthetized animals were injected with EB $20\,mg \cdot kg^{-1}$ in the caudal vein 10 min before sacrifice and EB dye was extracted in formamide from selected organs collected after exsanguination.
Results: GW825964X reduced the plasma insulin-to-glucose ratio by 46.4% ($p<0.03$) and increased plasma GLP-1 concentrations slightly (Δ=+33.8%; $p=0.16$) relative to the control group. There was no effect of GW825964 on body weight, mean arterial blood pressure or plasma triglyceride concentrations ($p>0.05$). GW825964X did not affect extravasation of EB in any of the following organs: skeletal muscles (*rectus femoris*, *soleus*, *gastrocnemius lateralis*, *vastus lateralis* and *tibialis cranalis*), heart, kidney, liver, lung or spleen ($p>0.05$). Neither plasma nitrite (NO_2^-) levels nor plasma VEGF concentrations were significantly affected by GW825964X compared to the vehicle-treated animals.
Conclusion: A 4-week treatment with GW825964X, a potent and selective DPP-IV inhibitor, does not impact the regulation of tissue microcirculation and/or permeability. This suggests that presumed changes in the metabolism of vasoactive peptides do not appear to be physiologically relevant *in vivo* in the whole animal.

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Effect of MK-0431, a dipeptidyl peptidase IV (DPP-IV) inhibitor, on glycemic control after 12 weeks in patients with type 2 diabetes

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Background and Aims: MK-0431 is an oral, potent, and selective DPP-IV inhibitor being developed to treat type 2 diabetes.
Materials and Methods: This was a randomized, double-blind, placebo-controlled, parallel group, dose-range finding study in patients with type 2 diabetes. After an initial diet/exercise phase and, for those on an antihyperglycemic agent, a drug wash off period, 552 patients aged 30–74 yrs who had HbA1c levels of 5.8 to 10.4% were randomized to one of 5 treatments: placebo; MK-0431 25, 50, or 100 mg q.d.; or MK-0431 50 mg b.i.d. for a 12-wk treatment period.
Results: Mean baseline HbA_1c levels ranged from 7.6–7.8% across treatment groups, with 28.8% of patients ≤7%. The efficacy analysis was based on a modified intention-to-treat population using an ANCOVA. After 12 wks, treatment with all MK-0431 doses significantly reduced HbA_1c compared to baseline, with the largest reductions observed in the 100 mg q.d. group. Across the MK-0431 dose range studied, differences in placebo-subtracted HbA1c ranged from -0.4% (25 mg q.d. group) to –0.6% (100 mg q.d. group) for the last observation carried forward (LOCF) analysis, and from -0.4 to –0.7% for the per protocol analysis without data carried forward (non-LOCF). Observed HbA_1c differences from placebo were greater with higher baseline HbA_1c: in the 100 mg q.d. group, patients with baseline HbA_1c <7% had a difference of -0.4% for LOCF and -0.3% for non-LOCF; patients with baseline HbA_1c 7–8.5% had a difference of –0.6% for both LOCF and non-LOCF; patients with baseline HbA_1c 8.5–10% had a difference of -0.8% for LOCF and -1.1% for non-LOCF. Fasting plasma glucose increased by 0.01 mmol/L in the placebo group, and dose-dependently decreased by 0.59 to 0.94 mmol/L in the other treatment groups. A similar reduction was also observed for other glycemic endpoints including fructosamine and mean daily glucose. Only one hypoglycemia adverse event was reported in each MK-0431 treatment group. No mean change in body weight was observed.
Conclusion: MK-0431 monotherapy was efficacious and generally well-tolerated in the treatment of patients with type 2 diabetes in this study.
Study sponsored by Merck & Co. Inc.

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Twelve-week efficacy and tolerability of MK-0431, a dipeptidyl peptidase IV (DPP-IV) inhibitor, in the treatment of type 2 diabetes

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Background and Aims: MK-0431 is an oral, potent, and selective DPP-IV inhibitor currently under development for the treatment of type 2 diabetes.

Materials and Methods: In a randomized, double-blind, placebo-controlled, active-comparator, parallel group, dose-range finding study, MK-0431 was evaluated in patients with type 2 diabetes. After an initial diet/exercise phase and, for those on an antihyperglycemic agent, a drug wash off period, 743 patients aged 21–76 yrs who had HbA1c levels of 6.3 to 11.0% were randomized to one of 6 treatments: placebo; MK-0431 5, 12.5, 25, or 50 mg b.i.d.; or glipizide 5 mg (up-titrated to 10, 15, and then to 20 mg/day) for a 12-wk treatment period.
Results: Mean baseline HbA_1c levels ranged from 7.8–7.9% across treatment groups, with 20.8% of patients ≤7%. The efficacy analysis was based on a modified intention-to-treat population using an ANCOVA. At Week 12, treatment with all MK-0431 doses significantly reduced HbA1c compared to baseline with the largest reductions in the 50 mg b.i.d. group: the placebo-subtracted differences in HbA1c ranged from -0.4 to -0.8% in a dose-dependent manner for the MK-0431 treatment groups, and -1.0% with glipizide. At Week 12, placebo-subtracted HbA1c results did not appear to have reached a plateau in the active treatment groups. FPG increased by 0.44 mmol/L in the placebo group, and dose-dependently decreased by 0.04 to 1.01 mmol/L in the MK-0431 groups, and by 1.38 mmol/L in the glipizide group. A similar reduction was also observed for other glycemic endpoints including fructosamine and mean daily glucose. Treatment with MK-0431 was well tolerated and resulted in no significant weight change, whereas a 1.1 kg weight gain was observed in the glipizide group. Twenty-one patients (17.1%) in the glipizide group experienced one or more hypoglycemic events compared to 3 (2.4%) patients in the placebo group and 0, 5 (4.1%), 5 (4.1%), and 2 (1.6%) patients in the MK-0431 5, 12.5, 25, 50 mg b.i.d. groups, respectively.
Conclusion: In this study, MK-0431 monotherapy was efficacious and generally well-tolerated in the treatment of patients with type 2 diabetes. The study is continuing with an active-controlled treatment phase to address the comparative efficacy, durability, β-cell function, and safety with longer term treatment.
Study sponsored by Merck & Co. Inc.

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Improved glycemic control and reductions in body weight in exenatide-treated subjects with type 2 diabetes with modest hyperglycemia
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Background and Aims: Exenatide is an incretin mimetic that exerts glucoregulatory actions in patients with type 2 diabetes mellitus (DM2). An interim data summary assessed the effects of 34 weeks of exenatide treatment on HbA_{1c} and body weight in subjects with DM2 under reasonably good glycemic control.
Materials and Methods: Subjects (N=87) using metformin (MET, n=68) or diet and exercise (DE, n=19) to control their DM2 were randomized to a triple-blind, placebo (PBO)-controlled study, receiving 4 weeks of treatment with PBO or exenatide (2.5, 5, 7.5 or 10 μg) administered subcutaneously (SC) BID. Subsequently, subjects continued in an open-label uncontrolled extension study, during which they received 5 μg exenatide SC BID for 4 weeks, followed by 10 μg exenatide SC BID. Results are presented for subjects who completed 30 weeks in the open-label study (34 weeks in total), with baseline set at Week 0 of the initial study (55% female, age 52 ± 11y, weight 100 ± 18 kg, BMI 35 ± 6 kg/m^2, HbA_{1c} 7.5 ± 0.7%, diabetes duration 4 ± 5y, mean±SD). Subjects using MET continued to do so throughout the study.
Results: Exenatide reduced HbA_{1c} from baseline at Week 34 (-0.9 ± 0.1%, mean±SE), with 67% and 49% achieving HbA_{1c} ≤7% and ≤6.5%, respectively. Body weight was also reduced (-3.8 ± 0.5 kg, mean±SE). Comparable changes were noted in the MET and DE treated subgroups. Specifically, the exenatide + DE subgroup had a mean (±SE) HbA_{1c} change of -1.0 ± 0.2% from baseline (7.6%), with 60% and 44% achieving HbA_{1c} ≤7% and ≤6.5%, respectively. Body weight changes of -4.3 ± 1.3 kg (mean±SE) from baseline (106 kg) were also observed. Likewise, the exenatide + MET group had a mean (±SE) HbA_{1c} change of -0.9 ± 0.1% from baseline (7.5%), with 69% and 51% achieving HbA_{1c} ≤7% and ≤6.5%, respectively. The mean (±SE) body weight change from baseline (99 kg) was -3.7 ± 0.5 kg. The most frequent adverse event was mild-to-moderate nausea. There were no cases of severe hypoglycaemia, with a low incidence (6%) of mild-to-moderate hypoglycaemia.
Conclusion: A robust lowering of glycaemia (HbA_{1c}) and body weight was observed in a group of subjects with DM2 treated with exenatide. This effect is notable given the early stage of the disease and modest hyperglycaemia (baseline HbA_{1c} ~7.5%) in these subjects, suggesting that exenatide can exert its effects early in the disease continuum.

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Reduced glycemic variability and risks for hypoglycemia and hyperglycemia with exenatide therapy as compared to insulin glargine
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Background and Aims: Glucose fluctuations affect cognitive function, and predict risks of significant hypoglycemia or hyperglycemia not captured by measures of average glycemia or HbA_1c. Since incretin mimetics exert a robust glycemia lowering effect that includes a reduction in meal excursions, we tested whether blood glucose (BG) variability analysis reveals therapy effects of the incretin mimetic exenatide over and above that observed by HbA_1c.
Materials and Methods: Methods (Kovatchev, Diabetes Technology and Therapeutics, 2002) analyzing glycemic variability and extremes were used post-hoc with self-monitored BG from a randomized, open-label study (GWAA) comparing insulin glargine and exenatide for 26 weeks. Patients were randomized to exenatide (5 μg BID for first 4 wks, 10 μg BID for remainder of study, n=282) or glargine QD (n=267), adjunctive to pre-existing MET+SU. Insulin glargine subjects started 10 U/day titrating to BG < 5.6 mmol/L unless hypoglycemic. Seven point glucose profiles were done twice at baseline and 10 times on therapy. Analytical measures included BG change pre-to-post-meal (BG variability), the high BG index (HBGI) and the low BG index (LBGI), characterizing hyperglycemic and hypoglycemic risk respectively. Data are given as mean (SD).
Results: The BG change (mmol/L) pre- to 2 h post-meal was smaller at breakfast [.96(1.6) for exenatide vs. 2.5(1.8) for insulin glargine] and at supper [.09(1.7) for exenatide vs. 2.0 (1.7) for insulin glargine], a 2.5-fold and 23-fold respective decrease, both $p < .001$. Overall mean BG (mM) [8.5(1.7) for exenatide vs. 8.4(1.6) for insulin glargine, p=.97] and final HbA_1c [7.2 for both, p=.69] did not differ. Reducing hyperglycemia may lead to hypoglycemia; thus LBGI was compared overall, pre-meal and at 3 am for both treatments. Overall, LBGI was .54(.62) for exenatide vs. .72(.78) for insulin glargine, $p = .003$, indicating a lower risk of hypoglycemia associated with exenatide treatment. Pre-meal, LBGI was .53(.75) for exenatide vs. .98(1.0) for insulin glargine, $p < .001$; at 3 am it was .61(1.0) for exenatide vs. 1.3(1.9) for insulin glargine, $p < .001$. Hypoglycemic risks were low for both therapies, but reduced by ~50% for exenatide. HBGI was not significantly different for the two therapies overall [5.5(4.7) for exenatide vs. 5.8(4.3) for glargine, $p = .4$], but reduced for exenatide post breakfast [7.4(6.2) for exenatide vs. 9.1(6.7) for insulin glargine, $p = .002$] and after supper [5.6(5.1) for exenatide vs. 9.0(6.7) for insulin glargine, $p< .001$]. Analysis of the final 13 weeks alone confirmed main findings. Covariate analysis suggests BG change pre- to post-meal was independent of HbA1c.
Conclusion: Average glycemia was identical for both groups, but exenatide reduced BG variability and hyperglycemia risk at meals. Hypoglycemia risk was low with both treatments, but was lower for exenatide, particularly during the night. Despite equal HbA_1c and average BG, analysis of variability and BG extremes shows substantial differences between treatments. Future studies would help determine whether these effects reduce the negative symptomatic and cognitive consequences of postprandial glucose excursions.

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Exenatide maintains glycemic control for 2 years in patients with type 2 diabetes: data from an ongoing, open-label study
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Background and Aims: Type 2 diabetes manifests with progressive deterioration in β-cell function and a progressive worsening in glycemic control despite use of currently available therapies. Exenatide is an incretin mimetic that, when added to a background of metformin (MET), sulphonylurea (SU), or a combination of both agents, has been shown to improve glycemic control and cause reductions in weight in patients with type 2 diabetes.
Materials and Methods: This interim 2-year analysis of an open-label extension of the original pivotal trial cohort involving subjects with type 2 diabetes treated with MET, SU, or a combination of both, examined the effects of twice-daily exenatide treatment (10 μg BID) on glycemic control (HbA_{1c}), safety, and tolerability. In the pivotal trial, exenatide was subcutaneously administered in a BID regimen before breakfast and supper. Doses were initiated at 5 μg BID and increased to 10 μg BID after 4 weeks for the

remainder of the 30-week, controlled trial. At the beginning of the open-label study, all subjects received 5 µg BID and were then titrated to 10 µg BID after 4 weeks. At the time of the interim analysis, 195 subjects (124 M, 71 F; age 56.5 ± 9.5y; BMI 34.0 ± 5.7 kg/m^2; mean±SD; baseline HbA_{1c} 8.2 ± 0.1%; baseline fasting plasma glucose (FPG) 9.4 ± 0.2 mmol/L; mean±SE) had completed 2 years of exenatide treatment (completer population).
Results: Subjects completing 2 years of exenatide treatment had reductions in HbA_{1c} and FPG from baseline of -1.2 ± 0.1% and -1.1 ± 0.2 mmol/L (mean±SE) at 12 weeks, respectively. After 2 years, changes in glycemic control were durable, with reductions in HbA_{1c} and FPG from baseline of -1.1 ± 0.1% and -1.1 ± 0.2 mmol/L (mean±SE), respectively. Furthermore, 41% of subjects achieved HbA_{1c} ≤7%. Exenatide was generally well tolerated during the open-label, uncontrolled extension study, the most frequent adverse events were gastrointestinal in nature.
Conclusion: In this ongoing open-label extension study, exenatide exerted durable improvements in glycemic control out to 2 years in subjects with type 2 diabetes treated with MET, SU, or combined MET/SU.

PS 67
Various novel therapeutic agents

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Beneficial effects of new antioxidant L-2264 on metabolic disturbances in rats with fructose-induced insulin resistance
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Background and Aims: Increased oxidative stress is believed to be one of the mechanisms responsible for macrovascular complications in insulin resistance states. We have previously shown that the new derivative of triazolopirimidines L-2264 decreases lipid peroxidation and improves lipid profile in diabetic rabbits. The aim of the study was to determine the effect of L-2264 on insulin resistance and anti/prooxidant status in fructose-fed rats.
Materials and Methods: Male Wistar rats were divided into three groups (8 animals each): the control group (C), the high fructose-fed group (F), which had free access to 250 g/L solutions of fructose for 8 weeks and fructose-fed group treated with L-2264 (100 mg/kg/day per os) for 8 weeks. At the end of the study fasted rats were subjected to the glucose tolerance test (GTT, 3 g/kg i.p.). Fasting glucose and insulin concentrations were used to calculate the homeostasis model assessment index of insulin resistance (HOMA-IR). Serum levels of NEFA, triglycerides (TG) and activity of hepatic glucose-6-phosphatase were measured as parameters of insulin resistance. Oxidative status was estimated by thiobarbituric acid reactive substances (TBARS), hydroperoxides (conjugated dienes and tetraenes) in liver and serum total antioxidant activity (TAA).
Results: Basal glycaemia was not different between all group, but, compared to control rats, fructose feeding for 8 weeks induced insulin resistance (HOMA-IR index F: 4.67 ± 0.27 vs C: 1.48 ± 0.01, $p<0.001$) and glucose intolerance ($p<0.001$) in rats. Administration of L-2264 increased insulin sensitivity (HOMA-IR index 3.19 ± 0.25, $p<0.01$) and glucose tolerance (area under curve over GTT was 699 ± 53 vs F: 992 ± 61 mmol/l/min, $p<0.01$) compared to F group. Furthermore, plasma TG, NEFA levels and glucose-6-phosphatase activities were higher in F group than in control group ($p<0.01$). L-2264-supplementation ameliorated all of these metabolic defects decreasing TG concentration (0.603 ± 0.013 vs F: 0.896 ± 0.043 mmol/l, $p<0.001$), NEFA levels (1.80 ± 0.11 vs F: 4.14 ± 0.25 mmol/l, $p<0.001$) and activity of key enzyme of gluconeogenesis - glucose-6-phosphatase in liver (15.48 ± 0.61 vs F: 32.25 ± 1.05 µmol/g·min, $p<0.001$) compared to F group. Moreover, high fructose consumption disturbed anti/prooxidant balance, as indicated by the significant higher levels of TBARS ($p<0.001$), hydroperoxides ($p<0.02$) and lower TAA ($p<0.001$) compared with control animals. The use of L-2264 was resulted in inhibition lipid peroxidations: a reduction of TBARS (49.93 ± 3.54 vs F: 105.46 ± 4.98 µmol/g, $p<0.001$), conjugated dienes (84.49 ± 4.79 vs F: 116.31 ± 12.11 µmol/g, $p<0.01$) and tetraenes (21.49 ± 1.81 vs F: 30.39 ± 3.32 E/g, $p<0.01$) levels in comparison with F group. Furthermore, TAA was increased (49.67 ± 3.37 vs F: 37.67 ± 2.74%, $p<0.02$) after administration of L-2264.
Conclusion: We revealed that treatment with L-2264 ameliorates metabolic impairments in rats with fructose-induced insulin resistance decreasing glucose intolerance, hyperinsulinemia, hypertriglyceridemia, NEFA levels, gluconeogenesis and lipid peroxidation. These data justify the perspectivity of L-2264's future studies as agent for the treatment of insulin resistance state.

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Antioxidant capacity of a prescribed Chinese traditional medicine preparation and its effects on microvascular endothelial cells in high glucose milieu
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Background and Aims: According to the pathogenesis that general inflammation and endothelial dysfunction were implicated in diabetic vascular complications, a Chinese Traditional Medicine preparation (CTMP) was prescribed to early prevent and manage diabetic vascular complications. This study was to determine the antioxidant capacity of the preparation and its each constitutional material and to investigate its effect on microvascular endothelial cells in high glucose milieu and its possible antioxidant mechanism.

Materials and Methods: Hydroxyl radicals were generated in a model system in vitro in which Fenton reactions took place, then the radical eliminating effect of the prescribed Chinese Traditional Medicine preparation and its each constitutional material was measured by electron paramagnetic resonance (EPR) method. Pulmonary microvascular endothelial cells of SD rat were cultured in medium with 5.56 mmol/L glucose (NG group), 20 mmol/L glucose (HG group), 20 mmol/L glucose and CTMP with working concentration at 1:100 in volume, and 20 mmol/L glucose with 10 mmol/L N-acetyl-L-cysteine (NAC group) respectively. Cells growth status was evaluated with the following procedures: microscopic morphology, vitality determination with Trypan blue staining and MTT (Thiazolyl blue) chromatometry. Rat gene chips from Affymetrix Corporation were applied to analyze the gene expressions in the four groups.
Results: EPR determination revealed that CTMP could extinguish 82.5% of the radicals generated in the model system and the scavenging capacity of single component medicine ranged from 16.7% to 46.2%. Compared with normal glucose concentration, high glucose concentration inhibited endothelial cell proliferation obviously. Cell growth, however, restored to normal by adding CTMP or NAC into high glucose medium and the effect of CTMP was similar to those of NAC. Gene chip analyses showed that 22 genes in HG group, compared with genes in NG group, were down-regulated and 9 genes up-regulated with a no less than 2-fold ($\log_2$ transformed) change in amplitude (Wilcoxon signed rank test, $p>0.998$, $p<0.0025$ respectively). Compared with those in HG group, 47 genes in CMTP group and 46 genes in NAC group underwent a more than 2-times amplitude change in expression, with 36 and 37 genes down-regulated, 11 and 9 genes up-regulated in CTMP group and NAC group respectively. Among those genes up-regulated or down-regulated with a more than 1.5-fold change in amplitude in both CMTP group and NAC group, two genes_ATP synthase H+ transporting, mitochondrial F0 complex, subunit b, isoform gene (Probe Set ID: rc_AA799778_at) and cytochrome b5 gene (Probe Set ID: rc_AA945054_s_at), which are associated with electron transporting on respiratory chain in mitochondria,were observed to be up-regulated. And this alteration might lead to reduction of mitochondrial superoxide production.
Conclusions: The CTMP has a potent capacity of eliminating oxygen radicals in vitro and can improve microvascular endothelial cells growth in high glucose medium with similar effect to NAC. The CTMP's ability to scavenge oxygen radicals and possibly diminish mitochondrial oxygen radicals production might involved in its protective effect on endothelial cells in high glucose surroundings.

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SGL0010, a novel orally active inhibitor of sodium-dependent glucose cotransporter, improves hyperglycemia through the enhancement of glucose excretion in Zucker diabetic fatty rats

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Background and Aims: In the kidney, plasma glucose is continuously filtered in the glomeruli and then reabsorbed in the proximal tubules by a class of transporters called the sodium-dependent glucose cotransporters (SGLTs). Two subtypes of SGLTs exist mainly in the renal proximal tubules, a low-affinity/high-capacity SGLT2 and a high-affinity/low-capacity SGLT1. Inhibition of these SGLTs in the kidney could result in suppression of glucose reabsorption and cause therapeutically useful enhancement of urinary glucose excretion. We have recently reported that SGL0010, a novel orally active SGLT inhibitor, reduced blood glucose excursion following an oral glucose tolerance test through the enhancement of urinary glucose excretion in obese male Zucker fa/fa rats. The aim of this study was to further characterize the antihyperglycemic effect of SGL0010 especially in type 2 diabetic animal model Zucker diabetic fatty (ZDF) rats.
Materials and Methods: Cell lines stably expressing human SGLT2 or SGLT1 and brush border membrane vesicles (BBMV) prepared from kidney of Sprague-Dawley rats, beagle dogs and humans were used for measurement of sodium-dependent glucose uptake. Male ZDF rats (13 weeks of age) were dosed orally with SGL0010 (1, 3, 10 and 30 mg/kg). Blood samples were collected under anesthesia before and 0.5, 1, 2, 4, 6, 8 and 24 hours after administration of SGL0010. Urine samples were collected for 8 hours after administration of SGL0010 using metabolic cages. Glucose contents in both plasma and urine were measured with a commercially available kit.
Results: SGL0010 inhibited SGLT activities in SGLT1 and SGLT2 expressing cells at IC_{50} of 0.923 μmol/L and 0.162 μmol/L, respectively. Also, SGL0010 inhibited SGLT activities in renal BBMV derived from rats, dogs and human (IC_{50}=0.342, 0.587 and 0.553 μmol/L, respectively). In ZDF rats, the significant enhancement of urinary glucose excretion was observed by single oral administration of SGL0010 at the doses from 3 mg/kg (2085 mg) to 30 mg/kg (2207 mg) compared to the vehicle control group (1143 mg). In addition, plasma glucose levels dose-dependently decreased after administration of SGL0010 (3 to 30 mg/kg) in the non-fasted state. It decreased from 491 ± 24 mg/dL to 392 ± 13 mg/dL at 3 mg/kg, from 491 ± 24 mg/dL to 308 ± 15 mg/dL at 10 mg/kg, and from 492 ± 23 mg/dL to 262 ± 21 mg/dL at 30 mg/kg, respectively. The area under the curve for plasma glucose (~8 hrs) [Glucose$AUC_{0-8\,hr}$] was significantly lowered by SGL0010 at the doses from 3 mg/kg (21.8% reduction) to 30 mg/kg (44.7% reduction) compared to the vehicle control group.
Conclusion: A novel and orally active SGLT inhibitor, SGL0010, improves hyperglycemia as a result of increase in urinary glucose excretion related to the suppression of glucose reabsorption in the renal proximal tubules in male ZDF rats. These efficacy in type 2 diabetic animal models suggest that SGL0010 appears to have a therapeutic potential in the new class of antihyperglycemic agents.

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Antidiabetic effects of SGL0010, a novel orally active inhibitor of sodium-dependent glucose cotransporter, in db/db mice

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Background and Aims: SGL0010, a novel sodium-dependent glucose cotransporter (SGLT) inhibitor, potently inhibits human SGLT1 and SGLT2 activities (IC_{50}=0.923 and 0.162 μmol/L, respectively). In the oral glucose tolerance test using animal models, an oral administration of SGL0010 enhanced urinary glucose excretion by inhibiting glucose reabsorption in renal proximal tubules. In the present studies, the anti-diabetic effects of SGL0010 were examined in spontaneously obese and type 2 diabetic db/db mice in the single and the chronic administration experiments.
Materials and Methods: In vitro activity of sodium-dependent glucose uptake via SGLT in renal brush border membrane vesicles (BBMV) of db/db mice was assayed. Urinary glucose excretion and plasma glucose level were measured in db/db mice (7 weeks of age, male) after single oral administration of SGL0010. In the chronic administration study, SGL0010 was given to db/db mice twice daily for 4 weeks (7- to 11 weeks of age, male). Plasma glucose and insulin levels were monitored weekly under non-fasting condition. Haemoglobin A1c (HbA_1c) was measured before and after the chronic treatment. Pancreatic insulin content and histopathological insulin positive area (% of islet) were measured after the chronic treatment.
Results: SGL0010 inhibited SGLT activities in renal BBMV derived from db/db mice at IC_{50} of 0.538 μmol/L. In the single oral administrations of SGL0010 in db/db mice, urinary glucose excretion for 8 hrs after the administration significantly increased at the doses of 10 and 30 mg/kg, respectively. Plasma glucose levels decreased immediately after the administration and the significant reductions in the area under the curve for plasma glucose (~8 hrs) [Glucose $AUC_{0-8\,hr}$] were observed at the doses of 3, 10, 30 and 100 mg/kg compared to the vehicle control group. In the vehicle control group, plasma glucose level increased from 402 ± 18 mg/dL to 678 ± 25 mg/dL, and plasma insulin level declined from 20.3 ± 1.6 ng/mL to 5.2 ± 0.8 ng/mL during the chronic administration experiment. The chronic treatment of SGL0010 (30 mg/kg, b.i.d.) suppressed plasma glucose level (522 ± 28 mg/dL in SGL0010 dosing group after 4 weeks treatment) and inhibited the decrease of plasma insulin levels (32.3 ± 6.9 ng/mL in SGL0010 dosing group after 4 weeks treatment). Also, chronic treatment of SGL0010 significantly suppressed the change of HbA_1c from the baseline value (Δ HbA_1c: 4.7% in the vehicle control vs. 2.2% in the SGL0010 group). Pancreatic insulin content was markedly lower in db/db mice than db/+m. The SGL0010 treatment inhibited the decrease of insulin content and increased insulin positive area (% of islet).
Conclusion: SGL0010, a novel orally active SGLT inhibitor, increased urinary glucose excretion via the inhibition of glucose reabsorption in the renal proximal tubules and improved hyperglycemia in db/db mice. Chronic administration of SGL0010 exhibited anti-diabetic effects in the same model. It is expected that this drug prevents exhaustion of pancreatic β-cells due to glucose toxicity. SGL0010 has a potent and attractive efficacy as an orally active anti-diabetic agent.

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A novel glucagon receptor antagonist, NNC 25-0926, blunts hepatic glucose production in the conscious dog

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Background and Aims: Elevated glucagon is associated with hyperglycemia during the fasting state in type 2 diabetes. New therapeutic agents that are capable of blocking glucagon's effect on hepatic glucose production (HGP) could be effective in lowering hyperglycemia in these patients. Our aim was to assess the ability of a novel glucagon receptor antagonist, NNC 25-0926 (NNC), to block glucagon's action on HGP in vivo.
Materials and Methods: Studies, performed using A-V difference and tracer techniques in 18 h fasted conscious dogs; consisted of equilibration (-140 to -40 min), control (-40 to 0 min) and experimental (EXP;0 to 180 min) periods. EXP was divided into period 1 (P1;0–60 min) and period 2 (P2; 60–180 min). In P1, vehicle or NNC was given intragastrically at 10, 20, 40 or 100 mg/kg as a bolus and euglycemia was maintained by glucose infusion as needed. In P2, somatostatin, basal intraportal insulin and 5-fold basal intraportal glucagon (2.5ng/kg·min) were infused.
Results: Arterial plasma insulin levels remained basal throughout the study in all groups (30 ± 12pM). Arterial plasma glucagon levels remained at basal levels during the CP and P1 and then increased by ~70 ng/l in P2 in all groups. Arterial plasma glucose levels were basal in CP and P1 in all groups. In response to a 5-fold rise in glucagon, plasma glucose increased to 13.6 ± 1.2 and 9.6 ± 0.6 mM in the vehicle and 10 mg/kg groups respectively, whereas in the 20, 40 and 100 mg/kg groups there was no rise in plasma glucose during P2. Net hepatic glucose output (HGO) was basal in all groups during the CP and P1 (~11.1 µmol/kg·min). In the vehicle group, net HGO increased by 40.4 ± 9.1 µmol/kg·min. This increase was blunted by NNC so that in the 10, 20, 40 and 100 mg/kg groups net HGO change from basal was 14.9 ± 7.7, 0.5 ± 2.6, 4.0 ± 3.1 and –3.9 ± 3.2 µmol/kg·min respectively. The ED_{50} was calculated as being 6.6 mg/kg. There was an approximately 2-fold higher systemic exposure to NNC compared to the hepatic portal vein, indicating a high degree of NNC uptake and clearance by the liver. Examination of the carbon flux indicated that the drug inhibited glycogenolysis.
Conclusion: NNC 25-0926 inhibits the ability of glucagon to increase plasma glucose by blunting HGP from glycogenolysis in a dose-dependent manner.
Support: Novo Nordisk a/s

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Somatostatin receptor subtype-2 agonist alleviates hyperglycemia in animal models of type 2 diabetes

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Background and Aims: Somatostatin (SST) inhibits glucagon and insulin release from pancreatic A- and B-cells. The effects of SST on its target cells are mediated via 5 pharmacologically distinctive SRIF receptor subtypes (sst1-sst5). In rodents pancreatic A-cells predominantly express sst2 whereas B-cells express mainly sst5. Glucagon significantly contributes to hyperglycemia in type 2 diabetes and immmunoneutralization of endogenous glucagon alleviates hyperglycemia. Agents that selectively suppress glucagon secretion are lacking. Recently, a highly sst2-selective non-peptidal agonist has been discovered. Aim of the study is to evaluate the potential use of the sst2 agonist in the therapy of type 2 diabetes.
Materials and Methods: Adult, male mice with genetic ablation of sst2, ob/ob, db/db mice and adult dogs were treated with a non-peptidal sst2-selective agonist. Isolated pancreatic islets were exposed to an sst2-agonist. Glucagon, insulin, glucose and growth hormone measurments were performed by ELISA or RIA.
Results: The sst2 agonist inhibited glucagon but not insulin release from isolated pancreatic islets and failed to inhibit glucagon secretion from islets obtained from sst2-deficient mice. The sst2 agonist lowered glucagon and glucose levels in wild type mice and failed to affect glucagon and glucose levels in animals with a deletion of the sst2-gene. In both animal models of type 2 diabetes the sst2 agonist decreased glucagon and glucose levels without affecting insulin. In healthy dogs, the sst2 agonist reduced overnight-fasted glucagon levels; glucose levels were reduced transiently, but hypoglycemia did not develop even at 10-fold higher doses. SST is a potent suppressor of growth hormone (GH) secretion, however the sst2 agonist did not influence circulating GH levels.
Conclusion: Since sst2 agonists do not interfere with insulin release judicious use of this class of compounds may prove effective in reducing insulin requirements early in type 2 diabetes reducing the need for insulin secretagogues. And finally, as small non-peptidal molecules, this class of compound provides the opportunity to develop orally active agents for the treatment of type 2 diabetes.
Support: DFG

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Effects of sibutramine in overweight poorly controlled Chinese female type 2 diabetic patients: a randomized, double-blind, placebo-controlled study

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Objective: To assess the efficacy of sibutramine 15 mg once daily as weight reduction in overweight and obese (BMI > 25 kg/m^2) Chinese female type 2 diabetic patients and to evaluate the influence of weight loss on diabetic control.
Methods: A randomized, double-blind, placebo-control, 12-week study was conducted. Chinese female type 2 diabetic patients, poorly controlled glucose levels, and HbA_{1C} > 8% were randomly assigned to two groups. In addition to their hypoglycemic agents (maximal doses of sulfonylureas and metformin), one group (N=30) received a sibutramine 15 mg once daily for 12 weeks and the other (N=30) received placebo for the same period.
Results: Comparing the changes that occurred after 12 weeks in the sibutramine and placebo groups, the former showed significantly greater reduction in body weight (2.5 vs. 0.1 kg, $P < 0.05$), fasting plasma insulin level (28.8 vs. 2.4 pmol/L, $P < 0.01$), 2-hr postprandial blood glucose after standard test meal (3.2 vs. 1.1 mmol/L, $P < 0.01$), insulin resistance (5.1 vs. 0.2, $P < 0.01$), HbA_{1C} (1.7% vs. 0.2%, $P < 0.05$), triglyceride (0.43 vs. 0.12 mmol/L, $P < 0.05$) and total cholesterol (0.52 vs. 0.08 mmol/L, $P < 0.05$). No significant differences were found between treatment groups in blood pressure and heart rate.
Conclusion: The addition of sibutramine to diet and oral hypoglycemic therapy resulted in significant weight loss and improvement in metabolic parameters in the treatment group. Sibutramine should be considered for use alongside diet and oral hypoglycemic therapy in Chinese overweight women with poorly controlled type 2 diabetes.

Table 1: Patient characteristics at baseline

Characteristics	Sibutramine	Placebo
Duration of DM (years)	9.12 ± 0.51	8.92 ± 0.62
Body weight (kg)	76.8 ± 2.1	78.3 ± 3.2
BMI (kg/m^2)	27.2 ± 1.1	26.9 ± 0.9
Fasting plasma glucose (mmol/L)	11.2 ± 0.4	12.1 ± 0.6
Postprandial plasma glucose (mmol/L)	15.7 ± 0.6	16.0 ± 0.4
Fasting plasma insulin (pmol/L)	109.2 ± 10.8	115.2 ± 6.6
HbA_{1C} (%)	9.8 ± 0.02	9.6 ± 0.01
HOMA-IR	11.9 ± 0.6	11.2 ± 0.3
Total cholesterol (mmol/L)	5.41 ± 0.2	5.46 ± 0.17
Triglyceride (mmol/L)	2.51 ± 0.11	2.44 ± 0.13
LDL-cholesterol (mmol/L)	3.55 ± 0.13	3.45 ± 0.16
HDL-cholesterol (mmol/L)	1.12 ± 0.05	1.17 ± 0.06
Uric acid (µmol/L)	345 ± 6	303 ± 30
Systolic blood pressure (mmHg)	131.2 ± 3.8	135.3 ± 5.1
Diastolic blood pressure (mmHg)	84.1 ± 2.1	85.6 ± 2.5
Heart rate (bpm)	76.2 ± 1.1	78.3 ± 1.6

Data are mean±SEM

Table 2: Comparison of the mean changes that occurred from baseline to 12 weeks in the sibutramine and placebo-treated groups

Characteristics	Sibutramine	Placebo	*P*
Body weight (kg)	2.5 ± 0.6	0.4 ± 0.3	< 0.05
BMI (kg/m^2)	1.6 ± 0.3	0.2 ± 0.2	< 0.05
Fasting plasma glucose (mmol/L)	3.4 ± 0.3	0.9 ± 0.1	< 0.01
Postprandial plasma glucose (mmol/L)	3.2 ± 0.6	1.1 ± 0.2	< 0.01
Fasting plasma insulin (pmol/L)	28.8 ± 3	2.4 ± 1.2	< 0.01
HbA_{1C} (%)	1.7 ± 0.01	0.2 ± 0.01	< 0.05
HOMA-IR	5.1 ± 0.7	0.2 ± 0.1	< 0.01
Total cholesterol (mmol/L)	0.52 ± 0.09	0.08 ± 0.05	< 0.05
Triglyceride (mmol/L)	0.43 ± 0.11	0.12 ± 0.04	< 0.05
LDL-cholesterol (mmol/L)	0.26 ± 0.08	0.2 ± 0.11	>0.05
HDL-cholesterol (mmol/L)	0.03 ± 0.03	0.02 ± 0.02	>0.05
Systolic blood pressure (mmHg)	5.1 ± 0.2	3.2 ± 0.1	>0.05
Diastolic blood pressure (mmHg)	3.2 ± 0.2	2.6 ± 0.2	>0.05
Heart rate (bpm)	7.3 ± 0.3	5.6 ± 0.5	>0.05

Data are mean±SEM

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Hypoglycaemia

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Oral aminoacids sustain cognitive function during insulin-induced hypoglycemia in type 1 diabetes mellitus

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Background and Aims: It has previously been reported that ingestion of a mixture of aminoacids (AA) limits the impairment of some aspects of cognitive function (CF), e. g. attention and memory, to insulin-induced hypoglycemia (H) in non-diabetic subjects.
Materials and Methods: To evaluate the potential effect of AA on CF in subjects with T1 diabetes mellitus (T1 DM), we studied 9 subjects (M/F 5/4, age 30 ± 2.8 years, BMI 23 ± 0.6 Kg/m^2, C-peptide < 0.2 nmol/L, HbA1c 7.4 ± 0.3%) on three different occasions, in random order, during intravenous insulin (2 mU/Kg/min) + variable glucose for 160 minutes. In two studies, clamped H (2.5 mmol/l for 40 min) was induced and either oral placebo (study P) or mixture of AA (42g) at +30 minutes (study AA) was given. The third study was as study AA, but euglycemia was maintained (study E). Symptoms and CF were measured at baseline (-30 and 0 min.) and at 120 min.
Results: Plasma glucose and insulin concentrations were no different in studies P and AA (p>0.2). After oral AA, plasma AA concentration increased to levels observed after mixed meal (2.4 ± 0.13 vs study P 1.7 ± 0.1 mmol/l, p=0.02). With the exception of glucagon (which increased more in study AA), counterregulatory hormones and total symptoms score were not different between AA and P. Beta-OH-butyrate was less suppressed after AA ingestion (200 ± 15 vs 93 ± 9 µmol/L, p=0.01), whereas plasma lactate was not different.. Among the cognitive tests compiled, the following indicated less deterioration after AA than P and E: Verbal memory, Trail-Making B, Digit span backward and PASAT (3 sec.) (P:-3.4 ± 1.3, AA:-1.2 ± 0.4, E:-0.1 ± 0.1, composite Z-scores, p<0.05 vs P).
Conclusions: oral AA ingested before H limit impairment of several cognitive aspects such as working memory, sustained attention and information processing. The less deterioration of cognitive tests after oral AA during H, in the absence of sensitive modifications of other alternative brain fuels, may be compatible with its use by some specific areas of the brain.
Support: JDRF 1-2002-102

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The impact on health-related quality of life (EQ-5D$_{index}$) in people with type 1 diabetes who experience severe hypoglycaemia

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Background and aims: The aim of this study was to evaluate the epidemiology and impact of Severe Hypoglycaemia (SH) in a UK population. Specifically, to determine the decremental impact of experiencing SH on Health-Related Quality of Life (HRQoL).
Materials and methods: A postal survey was mailed to 1,241 patients with type 1 diabetes. The survey included questions about frequency and impact of hypoglycaemia, diabetes management, lifestyle, diabetes-related complications and the EQ-5D. Episodes of SH events were self-reported. Detailed phenotypic details were available through the Health Outcomes Data Repository (HODaR). Multivariate regression modelling was utilised to quantify the relationship between SH and the EQ5D$_{index}$ standardised for potentially confounding factors. The resulting gradient coefficients were tested at the conventional level of 5%.
Results: 272 patients responded to the survey (21.9%); 49.1% were male. The mean age of respondents was 54.0 and 48.0 years for males and females, respectively. The overall mean frequency of self-reported SH was 2.2 events per person per year: 3.4 in females and 1.1 in males. The mean EQ-5D$_{index}$ of the respondents 0.72 (0.73 in females and 0.71 in males). The mean EQ-5D$_{index}$ for the subset of respondents who reported SH events was 0.58 (0.64 in females and 0.51 in males). After standardisation, the decrease in utility associated with people suffering one self-reported SH was 0.0121 units. Sex and BMI were not found to be significant predictors of utility in the model; however, included age, exercise level, number of complications and frequency of SH were.

Conclusions: Exposure to severe hypoglycaemia resulted in notably reduced quality of life. All measures should be taken to alleviate the impact of SH whilst maintaining good glycemic control.
Support: Novo Nordisk

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Effects of oral aminoacids on counterregulatory responses to insulin-induced hypoglycemia in type 1 diabetes mellitus
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Background and Aims: Oral amino acids (AA) potentiate glucagon responses to insulin-induced hypoglycemia (H) in non-diabetic subjects (N-DS). It is not known whether oral AA exert such an effect in subjects with T1 diabetes mellitus (T1 DM), who usually exhibit reduced/absent glucagon responses to H.
Materials and Methods: To establish the role of AA on counterregulatory (CR) responses in T1 DM, we studied 9 subjects (M/F 5/4, age 30 ± 2.8 years, BMI 23 ± 0.6 Kg/m^2, C-peptide < 0.2 nmol/L, HbA1c 7.4 ± 0.3%) on three different occasions, in random order, during intravenous insulin (2 mU/Kg/min) + variable glucose for 160 minutes. In studies 1 and 2 clamped H (2.5 mmol/l for 40 minutes from 120 to 160 minutes was induced and either oral placebo (study P) or mixture of AA (42 g) given at +30 minutes, (study AA) was given. The third study was as study AA, but in euglycemia (study E).
Results: Plasma glucose and insulin concentrations were not different during both H (p>0.2). Plasma CR hormones increased during H, however glucagon increased more in study AA (100 ± 16) vs study P (44 ± 8), and E (56 ± 7) (pg/ml, p=<0.05), mimicking responses previously observed in N-DS without AA (116 ± 15 pg/ml). However, glucagon response to AA was lower in T1DM than N-DS (318 ± 30 pg/ml, p<0.001). Adrenaline, noradrenaline and overall symptom responses were not different in AA and P studies. Glucose infusion rates were lower in AA than P studies (3.1 ± 0.7 vs 5.4 ± 0.4 mg/kg/min, p<0.01). After oral AA, plasma AA concentration increased to levels observed after mixed meal (2.4 ± 0.13, p=0.02 vs P:1.7 ± 0.1 mmol/l,). In T1 DM responses of glucagon to AA was highly correlated with that observed in P (r=0.82, p=0.006).
Conclusions: oral AA restore appropriate glucagon responses to H in T1DM and that the magnitude of these responses correlate with and can be predicted by, those occurring during H in the absence of AA.
Support: JDRF 1-2002-102

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Lack of effect of genetic variants in KCNJ11 and ACE genes on impaired hypoglycaemia awareness in patients with type 1 diabetes
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Background and Aims: Duration of diabetes, tight glycaemic control and repeated episodes of hypoglycaemia represent main risk factors of hypoglycaemia unawareness. However, even among patients with good glycemic control and longstanding type 1 diabetes awareness of hypoglycaemia may be intact. Genetic factors might explain some of this remaining variability.
Material and Methods: We studied the effects of genetic polymorphisms in the ACE (I/D) and KCNJ11 (Glu23Lys) (potassium inwardly-rectifying channel, subfamily J, member 11) on impaired hypoglycaemia awareness in 217 Caucasian type 1 diabetic patients. KCNJ11 encodes ATP-sensitive K+ channels, which are not only responsible for glucose sensing in the B-cell, but also in the hypothalamus. Hypoglycaemia awareness status was determined using standardised questionnaires and subjects were characterised for basic demographic and clinical parameters. The allele frequency for the ACE I/D polymorphism was 0.50 and 0.35 (Lys allele). Distribution of genotypes was in Hardy-Weinberg equilibrium for both polymorphisms (P=0.74 and P=0.57).
Results: In the univariate analyses, significant risk factors for impaired awareness of hypoglycaemia were C-peptide status, % HbA_1c, diabetes duration and age. In a logistic regression analysis, significant risk factors of impaired hypoglycaemia awareness were duration of diabetes, C-peptide and HbA1c (all P<0.01). No effects of I/D (P=0.30) or Glu23Lys (P=0.13) polymorphisms were observed in our study (adjusted for age, sex, diabetes duration, C-peptide and HbA1c).
Conclusions: A common genetic polymorphism in the KCNJ11 with functional relevance as well as the ACE I/D polymorphism do not seem to play a role for the risk to develop impaired awareness of hypoglycaemia. Diabetes duration, C-peptide and HbA1c represent risk factors for impaired hypoglycaemia awareness, which further indicates that the physiologic response to hypoglycaemia is worsening in course of the disease and intensive insulin treatment.

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Risk factors of severe hypoglycaemia and occurrence of silent hypoglycaemia in patients with type 1 diabetes assessed by continuous glucose monitoring
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Background and Aims: We assessed the relationship between conventional risk factors of severe hypoglycaemia and the occurrence of silent hypoglycaemia using continuous glucose monitoring.
Materials and Methods: A cohort of 119 patients with type 1 diabetes (age 46 years± 11 (mean±SD); diabetes duration 22 years± 12; HbA1c 8.3%± 1.1; 89% on multiple injections; 21% treated with ACE-inhibitor (ACEI) or AT2-receptor blockers (ARB)) participated in 6 days of continuous subcutaneous glucose monitoring with the Medtronic MiniMed Continuous Glucose Monitoring System (CGMS). Blood glucose measured with HemoCue 201+ was used to calibrate the CGMS 4 times daily. Participants kept a diary of meals, insulin dose and episodes of symptomatic hypoglycaemia. Primary endpoint was weekly number of symptomatic and asymptomatic hypoglycaemic episodes with CGMS-values ≤ 2.2 mmol/l. Endpoints were compared with known risk factors of severe hypoglycaemia and with ACEI/ARB treatment using multiple comparisons in analysis of variance.
Results: Total monitoring time was 714 days and valid monitoring time was 621 days (87%). Total number of episodes with CGMS-values ≤ 2.2 mmol/l was 3.9 per week including silent hypoglycaemia (3.0 per week), symptomatic hypoglycaemia (0.8 per week), and severe hypoglycaemia (0.06 per week). There was no relationship between the endpoints and known risk factors: HbA1c, awareness, diabetes duration, age, C-peptide status, ACE activity and late diabetic complications. Patients untreated with ACEI or ARB had 2.0 times more episodes of silent hypoglycaemia (p<0.01) than treated patients. After excluding patients on ACEI or ARB treatment we found 1.6 times more episodes of silent hypoglycaemia in patients with impaired awareness (p<0.01) than in patients with normal awareness.
Conclusion: Silent hypoglycaemia occurred three times weekly and was not related to known risk factors of severe hypoglycaemia. In patients treated with ACE-inhibitors or AT2-receptor blockers silent hypoglycaemia was half as frequent as in untreated patients.

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Recovery of cognitive function after insulin-induced hypoglycaemia in people with type 1 diabetes with either normal or impaired awareness of hypoglycaemia
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Aims: This study examined the time taken for restoration of cognitive function after hypoglycaemia in people with impaired and intact awareness of hypoglycaemia.
Methods: Thirty-six volunteers with type 1 diabetes underwent modified hyperinsulinaemic glucose clamps on two occasions. Twenty subjects had normal awareness of hypoglycaemia and 16 had impaired awareness. Arterialised blood glucose was initially maintained at 4.5 mmol/l for 30 minutes. During this time, a baseline test battery, consisting of the Edinburgh Hypoglycaemia Scale, four choice reaction time (CRT), Trail Making B (TMB) and Digit Symbol Substitution Test (DSST), were applied. Blood glucose was either maintained at 4.5 mmol/l throughout (euglycaemia), or lowered over 20 minutes to 2.5 mmol/l (hypoglycaemia), and maintained at this level for 1 hour. The test battery was repeated at the beginning and end of this experimental hour, after which euglycaemia (4.5 mmol/l) was restored. The order of hypoglycaemia and euglycaemia were randomised and counterbalanced. Subjects were blinded to the experimental condition. Testing took place every ten minutes during the recovery period and began after obtaining 2 consecutive glucose readings of >4.0 mmol/l. Test scores were compared using repeated measures ANOVA with hypoglycaemia and euglycaemia as within subjects factors and experimental order and state of awareness as between-subjects factors.
Results: In the normal awareness group, all cognitive tests showed significant impairment during hypoglycaemia (P<0.001 for CRT and DSST, P<0.05 for TMB). For DSST, no significant differences were observed dur-

ing recovery following euglycaemia and hypoglycaemia. For TMB, performance remained significantly impaired 10 minutes after hypoglycaemia (P=0.006). For mean CRT, performance remained significantly impaired (P=0.04) up to 40 minutes after hypoglycaemia. In the impaired awareness group, no significant impairment was observed during hypoglycaemia when compared with euglycaemia, so 'recovery' could not be assessed. Symptom scores were significantly higher during hypoglycaemia than euglycaemia (P=0.011 impaired awareness group, P=0.001 normal awareness group). The increment in symptoms during hypoglycaemia was significantly greater in subjects with normal awareness than in subjects with impaired awareness (P=0.020). Symptom scores returned to baseline immediately after hypoglycaemia, with no between-group differences thereafter.

Conclusions: In people with diabetes and normal awareness of hypoglycaemia, symptoms resolved immediately following hypoglycaemia but the recovery time for different domains of cognitive function varied considerably. There was no significant deterioration in cognitive function during hypoglycaemia in people with impaired awareness of hypoglycaemia, which may relate to cerebral adaptation in this group.

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Lower risk of hypoglycaemia with insulin detemir versus NPH insulin in elderly persons with type 2 diabetes: a pooled analysis of phase 3 trials

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Background and Aims: In general, elderly persons are more vulnerable to hypoglycaemia and its sequela due to reduced insulin clearance and associated co-morbidities. To evaluate if the benefits and risks of insulin detemir (IDet) vs NPH insulin (NPH) were similar in elderly (E; ≥ 65 years) and young (Y; < 65 years) persons, a pooled analysis of 3 multinational, open-label, randomised phase 3 trials in persons with type 2 diabetes was done.

Materials and Methods: A total of 418 E (IDet: N=239; NPH: N=179) and 890 Y (IDet: N=488; NPH: N=402) persons with type 2 diabetes, treated for 22–26 weeks with basal insulin plus meal-time insulin or oral agents were included in the analysis. Baseline characteristics were balanced between treatments and between elderly and young persons except for age (mean±SD: 70 ± 4 versus 55 ± 7 years) and disease duration (14 ± 8 versus 11 ± 7 years). HbA_{1c}, fasting plasma glucose (FPG), and weight were analyzed in an ANOVA model. Variation in fasting blood glucose (FBG) and relative risk (RR) of having hypoglycaemia (all events) were analysed using a mixed model and negative binomial models.

Results: HbA_{1c} and FPG at the end of the trial were similar between treatments and between elderly and young persons, while weight change, within-person variation in FBG, and RR of hypoglycaemia were lower with IDet than with NPH for both elderly and young persons. The incidence and pattern of adverse events were similar between treatments and between elderly and young persons.

	IDet (N)	Mean (SD)	NPH (N)	Mean (SD)	Difference (IDet-NPH) Mean	95% CI
HbA_{1c} (%) E	239	7.2 (0.8)	179	7.2 (0.8)	0.03	(-0.12; 0.18)
HbA_{1c} (%) Y	488	7.3 (0.9)	402	7.2 (0.9)	0.08	(-0.03; 0.20)
FPG* (mmol/L) E	192	8.0 (2.2)	134	8.0 (2.2)	0.05	(-0.44; 0.55)
FPG* (mmol/L) Y	353	8.2 (1.8)	255	8.0 (2.4)	0.19	(-0.20; 0.58)
Weight change (kg) E	239	0.6 (0.2)	176	1.6 (0.2)	-1.0	(-1.63; -0.44)
Weight change (kg) Y	480	0.8 (0.2)	395	2.0 (0.2)	-1.2	(-0.64; -0.75)
	N	SD (CV)	N	SD (CV)	p-value	
FBG** variation (mmol/L) E	236	1.28 (18.1)	176	1.71 (24.2)	<0.001	
FBG** variation (mmol/L) Y	480	1.22 (16.4)	395	1.58 (21.5)	<0.001	
Hypoglycaemia E	54% of subjects	1140 events	72% of subjects	1328 events	RR (IDet/NPH) 0.60	95% CI (0.42; 0.84)
Hypoglycaemia Y	53% of subjects	1708 events	58% of subjects	1733 events	RR (IDet/NPH) 0.77	95% CI (0.60; 0.98)

*FPG in 2 of 3 trials. **FBG in 2 of 3 trials

Conclusion: In conclusion, the benefits of insulin detemir – less hypoglycaemia, less within-person variation in FBG, and lower weight compared with NPH insulin – were seen in elderly as well as in young persons with type 2 diabetes without any increase in risk of hypoglycaemia.

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Memory function during hypoglycaemia in diabetic subjects with normal and impaired awareness of hypoglycaemia

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Aims: To study memory function during hypoglycaemia in people with type 1 diabetes who have normal and impaired awareness of hypoglycaemia.

Methods:

Subjects. 20 subjects with type 1 diabetes and normal self-assessed hypoglycaemia awareness (12 male, median HbA_{1c} 8.7%, median age 30 years) and 15 subjects with impaired hypoglycaemia awareness (6 male, median HbA_{1c} 8.2%, median age 34).

Experimental design. Each subject underwent two hyperinsulinaemic glucose clamps. Arterialized blood glucose was initially stabilized at 4.5 mmol/l, and subjects completed the learning phase of a novel video-based prospective memory task. Blood glucose was then lowered to 2.5 mmol/l (hypoglycaemia) or maintained at 4.5 mmol/l (euglycaemia) for one hour, during which the following tests were applied: (1) immediate verbal recall, using the Auditory Verbal Learning Test (AVLT); (2) immediate verbal logical recall, using the logical memory task from the Wechsler Memory Scales; (3) immediate visual recall, using the visual memory task from the Wechsler Memory Scales. The recall phase of the prospective memory task was completed during the experimental hour. Blood glucose was then restored to (or maintained at) 4.5 mmol/l, and after a further hour delayed recall for the verbal, logical and visual memory tasks was tested.

Results:

AVLT. Hypoglycaemia impaired immediate recall in aware subjects (euglycaemia 51.8 ± 7.8 vs hypoglycaemia 45.1 ± 9.4; P=0.003) but not unaware subjects (50.0 ± 15.2 vs 50.1 ± 10.1; P=0.347). Hypoglycaemia impaired delayed recall both in aware (10.0 ± 2.4 vs 8.3 ± 2.7; P=0.012) and unaware subjects (10.9 ± 3.7 vs 9.3 ± 3.4; P=0.017).

Logical memory. In aware subjects, hypoglycaemia impaired both immediate (15.6 ± 3.5 vs 13.1 ± 3.3; P=0.011) and delayed recall (13.1 ± 3.7 vs 11.0 ± 3.4; P=0.010). In unaware subjects there were no significant effects of hypoglycaemia (immediate recall: 17.2 ± 3.9 vs 16.2 ± 4.9, P=0.243; delayed recall 15.7 ± 4.5 vs 13.5 ± 4.3, P=0.053).

Visual memory. There were marked ceiling effects with this test, and no significant effects of hypoglycaemia were seen.

Prospective memory. Hypoglycaemia did not impair recall in the aware group (11.4 ± 3.9 vs 10.1 ± 4.9; P=0.121) but did in the unaware group (15.2 ± 3.9 vs 12.9 ± 4.8; P=0.020).

Conclusions: The hypoglycaemia-aware group suffered impairment of short-term verbal memory at 2.5 mmol/l, whereas the unaware group did not. This is consistent with cerebral adaptation in the unaware group. However, hypoglycaemia impaired delayed recall of the AVLT under euglycaemia in both groups. This suggests that cerebral adaptation in the unaware group improved short-term recall or attention, but not long-term memory formation. In the prospective memory task, learning took place during euglycaemia, so hypoglycaemia could only have affected long-term memory formation or recall. Thus, it again appears that long-term memory was susceptible to hypoglycaemia in the unaware group. It is not clear why the aware group had no deterioration under hypoglycaemia for the novel prospective memory task, and this aspect of memory deserves further attention.

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Hypoglycaemic episodes in children and adolescents with type 1 diabetes mellitus – an analysis of determinants and risk factors

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Background and Aims: Hypoglycaemia is a common adverse event of diabetes treatment. In addition to direct symptoms, hypoglycaemia adversely affects patients in many ways: It can cause fear and anxiety and is likely to increase health care resource use. The goal of the trial was to analyse hypoglycaemic episodes in children and adolescents with type 1 diabetes and to identify determinants and risk factors.
Materials and Methods: A cohort of 188 children and adolescents (age 12,7 ± 3,6 ys, diabetes duration 4.6 ± 3.6 ys, BMI 20.3 ± 4.3 kg/m^2, HbA1c 8.2 ± 1.5% [DCA 2000; mean normal 4.0–6.4%]), with type 1 diabetes consecutively admitted to our hospital was followed for one year by standardised questionnaires and reporting of episodes of hypoglycaemia.
Results: Using a questionnaire 126/188 patients (67%) were able to define precisely values below which they feel symptoms of hypoglycaemia (3.2 ± 0.7 mmol/l). In 24 patients the levels were very low (<2.5 mmol/l). Patients with low levels were older (15.2 ± 2.0 vs 13.2 ± 2.8 ys, p<0.001), were more lean (BMI 20.4 ± 3.8 vs 22.4 ± 4.4 kg/m2, p=0.041), performed more blood glucose self-tests/week (41.5 ± 8.5 vs 35.7 ± 9.1, p=0.006), but had higher HbA1c-levels (8.8 ± 1.5 vs 7.9 ± 1.3%, p=0.035). There were no significant differences between the groups in respect of the frequency of severe hypoglycaemia (with third party assistance) during the preceding 4 weeks (2 vs 3, p=0.307) or diabetes-related knowledge (20.4 ± 5.0 vs 20.1 ± 5.6 pts, p=0.864). Children which were not able to define a level below which they feel symptoms of hypoglycaemia were younger (11.3 ± 3.9 vs 13.5 ± 2.8 ys, p<0.001), performed less blood glucose self-tests/week (33.8 ± 7.6 vs 36.9 ± 9.1, p=0.039), but had more frequently symptomatic hypoglycaemic episodes during the preceding 4 weeks (13.1 ± 8.2 vs 10.7 ± 6.0, p=0.021). Performing ANOVA analysis, there were neither associations between the incidence of hypoglycaemia and HbA1c (p=0.20) or age (p=0.49) nor hypoglycaemia and diabetes-related knowledge (p=0.94). But, as most important factor to avoid hypoglycaemic episodes, patients' ability for hypoglycaemia awareness was identified.
Conclusion: In the pediatric age group hypoglycaemic episodes are frequent adverse events of diabetes treatment. In older children and adolescents poor hypoglycaemia awareness is associated with higher HbA1c, frequent self-monitoring and low BMI, possible indicators for psychological problems. Younger children without the ability to define values below which they feel hypoglycaemic symptoms are more likely to have hypoglycaemic episodes. Following these results age-dependent and specific treatment strategies are mandatory: 1. Psychological exploration and treatment in older children and adolescents with poor hypoglycaemia awareness. 2. Teaching and intensive training to improve the awareness of hypoglycaemic symptoms in younger children.

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An unusual presentation of hypoglycaemia: distal polyneuropathy caused by an insulinoma in the context of MEN type 1

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The usual underlying cause of spontaneous fasting hypoglycemia is the insulinoma, but peripheral neuropathy associated with hypoglycemia secondary to it, is unusual. The most recent papers report that among the thousands of patients treated of insulinoma, only 32 cases had hypoglycemic sensorimotor polyneuropathy. Here we present the case of young patient who presented polyneuropathy as the first manifestation of a multiple endocrine neoplasia type 1.
A 19-year-old woman had begun her disease in a progressive way, with symmetric weakness and wasting of hands and feet. She also complained of headaches, and on examination we found galactorrhea. The blood biochemistry showed severe basal hypoglycemia (11 mg/dl) in association with elevated levels of insulin (8.8 uUI/mL) and a C-peptide level of 1.2 ng/mL (normal range 0.9–1.4). The prolactin level was 1163 uUI/mL (normal range 72–511). The EMG showed sensorimotor polyneuropathy of the four limbs, predominantly distal. MRIs revealed a pituitary macroadenoma (2 cm), a tumor of 2 cm located in the tail of the pancreas and a mass of 1.7 cm in the left adrenal, which was nonfunctioning. Cortisol level was 16.16 ug/dL (normal range 6.2–19.4). We also found a PTH level of 147 pg/mL (normal range 15–65) with ionic calcium of 1.32 mg/dL (normal range 1.13–1.32). Sestamibi SPECT Scintigraphy revelead a parathyroid adenoma with rapid clearance of the tracer. With all of these features we concluded that she had multiple endocrine neoplasia type 1. The patient underwent a pancreatic caudal resection. The pathological examination revealed that the lesion was a benign islet cell adenoma. After the surgery, the patient had normal levels of glycemia (102 mg/dL) and insulin (7.1 uUI/mL). Now she is taking bromocriptine, and polyneuropathy has improved.
Peripheral neuropathy associated with hypoglycemia secondary to insulinoma in the context of a MEN type 1 is unusual. To our knowledge, this is the first published case of MEN type 1 that debuted with the compromise of peripheral nervous system. The literature suggests that hypoglycemia causes a phenomenon of ischemia/reperfusion in PNS. Some investigators proposed that the damage is morphological which explains the incomplete recovery after the pancreatic surgery, but others speculate that the origin of the damage is metabolic and functional. The experiments indicate that hypoglycemia is the mediator of neuronal damage due to disturbance of fast axonal transport, but it was hypothesized that insulin itself or other substances released by the tumor may damage the peripheral nerves.

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Changes of brain glucose transporter expressions to hypoglycaemia in streptozotocin-induced diabetic rats

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Background and Aims: Transport of glucose across plasma membranes is mediated mainly by Na^+-independent glucose transporters (GLUT). It has been reported that glucose transport into brain is specifically enhanced in chronically hypoglycemic rats. But, regulation of glucose transporter expression in the brain during diabetes remains controversial, and it has not been yet determined whether glucose transporters in the cerebral adaptation to insulin-induced hypoglycemia in diabetic rats can be changed. To investigate the role of brain GLUT1 and GLUT3 in the cerebral adaptation to chronic hypoglycemia in diabetic rats, we have investigated its expression in the non-diabetic and diabetic rat brain using RT-PCR and western blot analysis after hypoglycemic insults.
Materials and Methods: Adult male Sprague-Dawley rats weighing 250–300 g were housed in groups of three with ad libitum access to food and water. Rats were randomly assigned to become diabetic or to remain non-diabetic groups. Diabetes was induced by the intraperitoneal injection of streptozotocin (70 mg/kg). Hypoglycemia was induced by twice daily subcutaneous injection of intermediate-acting insulin with dosage adjustment according to the blood glucose levels. Mean daily capillary blood glucose reading performed during 5 baseline days and twice daily subcutaneous injections of intermediate-acting insulin (non-diabetic hypoglycemic group and diabetic hypoglycemic group) or saline (non-diabetic control

group and diabetic control group) during 6 days. At the end of the period of treatment, animals were decapitated and forebrains were removed and assayed by RT-PCR and western blot analysis.
Results: Mean blood glucose levels during 6 days of insulin treatment were 57.7 ± 10.2 mg/dL in non-diabetic hypoglycemic group, 310.9 ± 15.8 mg/dL in diabetic control group, 58.8 ± 9.1 mg/dL in diabetic hypoglycemic group, compared with 123.1 ± 9.4 mg/dL in non-diabetic control group. GLUT1 and GLUT3 mRNA expressions were not significantly changed in diabetic control group compared with those in non-diabetic control group. Also, hypoglycemic treatment had no significant influence on GLUT1 and GLUT3 mRNA expressions in non-diabetic hypoglycemic group. But, GLUT1 mRNA expressions in diabetic hypoglycemic group were significantly increased ($p < 0.05$) compared to diabetic control group. GLUT3 mRNA levels were not changed significantly compared with those in diabetic control group, but it showed a trend toward an increase in GLUT3 mRNA expressions. The level of GLUT1 protein was significantly increased ($p < 0.05$) in diabetic hypoglycemic group as assessed by western blot analyses compared to diabetic control group. But the level of GLUT3 protein was not changed in diabetic hypoglycemic group compared to diabetic control group.
Conclusion: These results suggest that brain GLUT1 may have an important role in cerebral adaptation to chronic hypoglycemia in diabetes and in hypoglycemia unawareness.

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Inhaled and oral insulin

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Abstract withdrawn

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Patient reported outcomes (PROs) using the Lilly/Alkermes Inhaled Insulin System versus injectable insulin in patients with type 1 diabetes (T1D)
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Background and Aims: Inhaled insulin delivery may lead to increased patient satisfaction and improved quality of life resulting in better treatment compliance and more favorable outcomes. The aim of this study was to assess the impact of the Lilly/Alkermes Inhaled Insulin System on PROs in T1D patients.
Materials and Methods: This randomized, open-label, crossover trial compared glycemic control (A1C) of mealtime Human Insulin Inhalation Powder (HIIP) with mealtime injectable (SC). Patient reported outcomes were assessed using the SF-36 Vitality Scale, Cognitive Distress, Fatigue, Hyperglycemia, and Hypoglycemia Subscales of the Diabetes Symptom Checklist-Revised, Diabetes Treatment Satisfaction Questionnaire and subscales of the Insulin Delivery System Questionnaire (IDSQ) (ie, overall satisfaction and preference, ease of dosing, and lifestyle impact). Instruments were administered to patients at baseline, crossover, and end of study. All IDSQ scales are scored 1-7 with higher scores corresponding to greater satisfaction.
Results: Patients (n=119) were 46% male with a mean age of 41 yrs and a mean baseline A1C of 8.1%. Patients receiving HIIP had significantly greater treatment satisfaction (30.0 vs. 27.2, $p < 0.001$) and greater insulin delivery system satisfaction (5.5 vs. 4.4, $p < 0.001$) compared with SC insulin. In addition, they agreed more strongly that HIIP could be incorporated into their lives (eg, facilitates travel, reduces embarrassment, and reduces reluctance to use pre-meal insulin) as compared to SC (5.6 vs. 5.1, $p = 0.002$). No other significant differences were found in PRO measures. Also, no significant treatment interactions by period were observed.
Conclusion: The Lilly/Alkermes Inhaled Insulin System uses a small, reliable, breath-actuated device to deliver HIIP to patients as an alternative to mealtime insulin injection. These results indicate that T1D patients may replace mealtime injections with HIIP without diminishing vitality, increasing the burden of diabetes symptoms, or lessening the ease of dosing. Moreover, treatment satisfaction is enhanced with this novel inhaled insulin system, which may facilitate treatment compliance with potential for better glycemic control.

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Markedly reduced postprandial glucose excursions through inhaled Technosphere/Insulin in comparison to sc injected regular insulin in subjects with type 2 diabetes
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Background and Aims: Technosphere® /Insulin (TI) is an encapsulated dry powder formulation of human insulin with a new, easy to use, drug delivery system (MedTone Inhaler) for pulmonary administration which has been shown to have a rapid onset of action and a duration of action long enough to cover meal-related glucose absorption. The primary objective of this study was to assess safety and efficacy of pre-prandially administered TI compared to subcutaneous (SC) regular insulin (RHI) on blood glucose (BG) concentration over a 7 day treatment period.
Materials and Methods: Sixteen non smoking subjects with type 2 diabetes (age 59 (range 39–69) ys; BMI 29.6 (23.8–34,9) kg/m^2; mean diabetes duration 12.3 ys; HbA1c 7.3 ± 0.9 (mean±SD) %) with normal pulmonary function (forced expiratory volume in 1 sec and forced vital capacity >80% of predicted normal) and treated with intensified insulin therapy were enrolled in this randomized, open-label, two period cross-over study. Subjects covered their prandial insulin needs either by inhaled TI or by SC RHI over a treatment period of one week, respectively, while continuing their

usual basal insulin therapy. The doses of TI and RHI were determined during a 24 h in-house period prior to randomization. TI was inhaled using a 12 U or 24 U cartridge via a hand-held inhaler (MedTone). After an outpatient period during which subjects administered the assigned pre-meal therapy with either SC or TI, performed 4-point BG self-measurements and pursued their usual activities and diet for 5 to 7 days, postprandial BG and serum insulin (INS) excursions were determined under in-house conditions after ingestion of a standardised breakfast (496 kcal, 55% carbohydrates) covered with doses of 48 ± 9 U of TI or 14 ± 5 IU of SC RHI. Samples were taken at baseline, and at 5, 10, 15, 30, 45, 60, 90, 120, 180, and 240 min after meal ingestion.
Results: Baseline adjusted postprandial total insulin exposure (INS-$AUC_{0-240\ min}$) was comparable for TI and for SC (8187 ± 4269 vs 8302 ± 4025 min*µU/dL; NS) whereas baseline adjusted postprandial glucose excursion (BG-$AUC_{0-240\ min}$) for TI was only about 50% of that of SC (5095 ± 5923 min*mg/dL vs 9851 ± 5593 min*mg/dL; p <0.008). With TI median insulin T_{max} was shorter (15 vs 120 min; p <0.001) and median C_{max} was higher (100 vs 54 µU/mL; p=0.001) than with SC. Accordingly, postprandial maximum adjusted BG excursions were lower with TI compared to SC (49 vs 82 mg/dL; p <0.003). The incidence of hypoglycaemia (BG <63 mg/dL or hypoglycemic symptoms) was comparable between TI and SC (6 vs. 5 episodes) as was the number of treatment emerged (mild to moderate) adverse events (5 vs. 4 episodes). Hyperglycaemia (BG >280 mg/dL) occurred more often with TI (12 vs. 4 episodes) – with two patients alone accounting for 8 episodes.
Conclusion: TI markedly improved post-prandial glucose control compared to prandial SC while total serum insulin concentration were comparable between both treatments. This is probably due to a rapid onset of action of TI with which insulin T_{max} resembles first-phase insulin release kinetics. In contrast SC insulin levels were much lower than TI during the early post-prandial period and did not exhibit the clear peak observed with TI. These results suggest that preprandial TI is superior to SC RHI in providing prandial insulin needs and reducing meal related blood glucose excursions.
This research was initiated and financed by MannKind Corp.

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Add-on therapy with Kos inhaled insulin is as efficacious as add-on therapy with Lantus® in poorly controlled type 2 diabetic patients treated with sulfonylureas or metformin
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Background and Aims: The safety and efficacy of Kos inhaled insulin administered by oral inhalation from a metered-dose inhaler was compared with bedtime SC Lantus (REF) injections in patients with type 2 diabetes poorly controlled with oral antidiabetic agents.
Materials and Methods: Twenty-four subjects with type 2 diabetes poorly controlled with sulfonylureas and/or metformin (5 females, 56 ± 6 years, BMI 33 ± 4 kg/m², HbA_{1c} 8.4 ± 0.9%) received either REF at bedtime or MDI up to 15 min before main meals as add-on therapy over 28 days. Blood glucose (BG) profiles were assessed under standardised conditions before and after 7 and 28 days of treatment.
Results (MDI vs. REF, Mean±SD): Both treatments improved metabolic control similarly. Mean BG change pre-treatment vs. post-treatment were −51 ± 34 vs. −43 ± 31 mg/dl, while mean postprandial BG values were −65.6 ± 43.3 vs. −43.5 ± 33.8 mg/dl. BG variability (expressed as mean amplitude of glycaemic excursions – MAGE) was −12.3 ± 19 vs. 0.4 ± 20 mg/dl with a slight trend in favour of the MDI treatment, (p=0.134). Patients receiving MDI showed slightly better improvements in overall diabetes control (HbA_{1c} −1.23 ± 0.52 vs. −1.05 ± 0.51%, fructosamine −58 ± 32 vs. −48 ± 39 µmol/l) than patients receiving REF. The mean change in LDL-cholesterol was −9.9% vs. +1.4% (p=0.122); and the change in serum triglycerides showed a statistically significant difference in favour of the MDI treatment (−35.8% vs. −11.5%, p=0.011). Confirmed hypoglycaemia (BG < 50 mg/dl) occurred 4 times with REF and 3 times with MDI. No difference in pulmonary adverse events was seen.
Conclusion: Mealtime inhaled insulin is as effective as the basal insulin analog Lantus in type 2 diabetic patients poorly controlled with oral agents. Inhaled insulin dosed using a user-friendly metered-dose inhaler would be an attractive alternative for these patients who refuse to be treated with s.c. insulin, despite poor metabolic control.
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Dose response of human insulin inhalation powder (HIIP) and dose equivalence to subcutaneous (SC) insulin lispro
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Background and Aims: Inhaled insulin may be a viable alternative therapy to multiple injections with subcutaneous insulin. The present study assessed the pharmacokinetic (PK) and glucodynamic (GD) dose-response of HIIP delivered using a system based on AIR® particle technology and its dose-equivalence to SC insulin lispro.
Materials and Methods: Twenty healthy, non-smoking male (N=10) and female subjects (age 29.6 ± 6.9 years; BMI 23.2 ± 2.3 kg/m²) with normal forced vital capacity and forced expiratory volume (FEV_1) received up to 4 single doses of HIIP (2.6, 3.6, 5.2, 7.8 mg) and 3 doses of SC insulin lispro (6, 12, or 18 U) in this open-label, randomised, seven-period crossover trial. Pharmacokinetic parameters were derived from serum insulin concentrations; GD parameters from glucose clamp data.
Results: Human Insulin Inhalation Powder demonstrated an extended time exposure and a prolonged duration of effect (late $T_{Rmax50\%}$: 412 vs 236 min, p<0.001) compared with SC insulin lispro. The HIIP versus lispro doses of 2.6 mg versus 6 U, 5.2 mg versus 12 U, and 7.8 mg versus 18 U, respectively, achieved similar overall PK $AUC_{(0\text{-}t')}$ and GD (G_{tot}) responses. The median insulin t_{max} was not different between HIIP and SC insulin lispro (45 for both), while the median time of return to baseline for PK was longer for HIIP compared to SC insulin lispro (480 vs 360 min). Intra-subject variability (PK parameters) was comparable between HIIP and SC insulin lispro (31 and 29%). Relative bioavailability and relative biopotency of HIIP were consistent across doses (8% and 9%). There were no statistically significant differences between the pre- and postdose FEV_1.
Conclusion: While the time-action profile was longer for HIIP than SC insulin lispro, both treatments showed a rapid initial absorption and similar overall PK exposure and GD effect. In addition, HIIP was as well tolerated as SC insulin lispro in healthy subjects, therefore offering a promising alternative to injectable insulin therapy.

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Impact of large tidal volume ventilation on absorption of inhaled insulin
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Background and Aims: Insulin is the cornerstone in the treatment of many patients with diabetes. Inhaled insulin seems to be a promising alternative to subcutaneously administered insulin. Little is however known about how different breathing patterns affect the absorption of inhaled insulin. Thus, the purpose of this study was to investigate the effect of large tidal volume ventilation (LTVV) on the absorption of inhaled insulin in rabbits.
Materials and Methods: Thirty-two (32) mechanically ventilated rabbits received a dose of inhaled human insulin (5U) via an UltraVent™ nebuliser system, and ventilation was adjusted according to randomization. There were four different treatment groups (N=8 each): 1) Normal tidal volume ventilation (NTVV) during the entire 120 min sampling period [NTVV], 2) LTVV during the entire sampling period [LTVV], 3) NTVV except for LTVV for 15 min starting immediately after dosing [Early LTVV], and 4) NTVV except for LTVV for 15 min starting at 60 min post dosing [Late LTVV]. Ventilation was controlled by means of pressure controlled ventilation with NTVV being 40 breaths/min and an inspiratory pressure of 10 cmH_2O over a positive end-expiratory (PEEP) of 2 cmH_2O, and LTVV being 20 breaths/min, and 23 cmH_2O over PEEP.
Results: The total insulin absorption ($AUC_{ins(0-120\ min)}$) was approximately 150% greater for [LTVV] compared to [NTVV] (2.49 [1.42;4.37] (Ratio [95%C.I.]), p<0.01). The maximal insulin concentration (C_{max}) for [LTVV] was approximately 105% greater than that of [NTVV] (2.06 [1.08;3.95], p=0.03), while the time to reach C_{max} (t_{max}) was not statistically significantly different for [NTVV] and [LTVV]. The appearance of insulin in the plasma was faster for [LTVV], with an initial rate of increase (best fitted line from 0 to 20 min) being approximately 120% larger for [LTVV] than for [NTVV] (2.18 [1.16;4.10], p=0.02). There was no difference in the terminal elimination rate constant for the two groups.
For [Early LTVV] and [Late LTVV], there were no statistically significant differences compared to [NTVV], however for the [Late LTVV] an increase

in insulin levels were observed after the LTVV period (n.s compared to NTVV), suggesting that deep breathing manoeuvres might induce an increase in the absorption of insulin even after a longer period of time.
Conclusion: Large tidal volume ventilation during the entire period had large effects on the absorption of inhaled insulin in rabbits, with a significantly increased $AUC_{ins(0-120\ min)}$ and C_{max}. These data could potentially have implications for patients using inhaled insulin in situations where a change in breathing pattern is seen, such as exercise.
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Addition of Oralin at meal-times in subjects with type 2 diabetes maintained on glargine + metformin - a comparison with placebo
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Background and Aims: The purpose of the study was to determine the metabolic effect of novel oral insulin spray formulation (Oralin) at meal-time, on a long term basis (12 weeks), in subjects with type 2 diabetes, maintained on once a day s.c. Glargine insulin injection therapy at bed time and Metformin. The primary hypothesis is that Oralin can be used safely at meal-times and will improve 7 point glucose profiles, fructosamine and the baseline HbA_1c levels 12 weeks after treatment. The Oralin system is based on a unique liquid aerosol formulation, which allows a precise insulin dose delivery by mouth.
Materials and Methods: This was a randomised, double blind, long term (12 weeks) study in 26 type 2 diabetic subjects (male or female) with poorly controlled blood glucose maintained on once a day s.c. Glargine + Metformin. After the initial screening visit, subjects maintained their regular treatment for two weeks as a run-in period. Following the training of the Oralin device operation and dosing schedules, they were divided into two groups. One group had 7 puffs of Oralin TID, and the other group had 7 puffs of placebo TID. Both groups took the puffs 10 min before meal time, in addition to their regular treatment. In cases where self glucose values were above 12 mmol/L before any meal or before bedtime, an additional 7 puffs were added. Each subject had routine blood chemistry and HbA_1c as well as fructosamine levels at the beginning of the study and at the end of every month during the study period. Beginning with the initial screening visit, each subject had to monitor his/her blood glucose at least three times a day and once a week for a 7-point profile.
Results: The interim results, after 8 weeks of treatment, showed no change in fasting glucose while in post-prandial glucose there was a 15.4% reduction (from 211.2 mg%± 53.7 to 178.5 mg%± 39.1) in the Oralin group versus 3.9% elevation (from 202.7 mg%± 60.1 to 210.1 mg%± 5.2) in the placebo group ($p<0.05$). Furthermore, we found a reduction of fructosamine in the Oralin group of 6.4% versus 3.6% in the placebo (p-NS) and in HbA1c - 6.6% reduction versus 3.4% in the placebo (p-NS).
Conclusion: In type 2 diabetic patients, maintained on Glargine and Metformin, Oralin was especially effective in controlling post-prandial glucose excursions.

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Biologic effectiveness of an insulin analogue developed for oral insulin delivery
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Background and Aims: Oral delivery of insulin could facilitate and potentially improve the treatment of diabetes, but it is associated with a number of challenges including bioavailability and reproducibility. To overcome those problems new insulin analogues are being produced. Insulin 105 (IN-105) developed by Nobex Corporation is such a molecule. The goals of the present studies were to compare the bioactivity of IN-105 to that of Humulin when given intravenously and to assess the reproducibility and pharmacokinetics of orally delivered IN-105.
Materials and Methods: *Experiment 1 (intravenous infusion):* Overnight fasted conscious dogs were studied. Following a 40 min control period, somatostatin was given to inhibit insulin and glucagon secretion, and glucagon was replaced intraportally in basal amounts. At the same time Humulin (n=6) or IN-105 (n=5) was given at 600 and 2000 μU/kg·min for two consecutive 120 min periods. Euglycemia was maintained in both groups by glucose infusion. *Experiment 2 (esophageal gavage):* In a separate set of studies to assess its oral effectiveness, IN-105 was given by gavage (0.25 mg/kg BW) in a liquid formulation (6.0 ml) to dogs (n=12) fasted for 42 h. Euglycemia was maintained by glucose infusion.
Results: *Experiment 1:* The arterial plasma insulin levels (pmol/l) were 78 ± 6 and 102 ± 18 (first test period) and 300 ± 30 and 360 ± 66 (second test period) with Humulin and IN-105, respectively. Net hepatic glucose balance switched from output to uptake of 0.8 ± 1.7 and 5.3 ± 1.1 μmol/kg·min in the first test period and 6.7 ± 3.9 and 10.0 ± 2.2 μmol/kg·min in the second test period with Humulin and IN-105, respectively. Likewise the glucose infusion rates (μmol/kg·min) required to maintain euglycemia were similar in the two groups during period one (14.4 ± 2.2, 18.3 ± 1.7) and period two (92.7 ± 11.6, 86.6 ± 8.9). *Experiment 2:* The arterial plasma insulin level rose from 42 ± 12 pmol/l to a peak of 258 ± 54 pmol/l at 10 min and fell to baseline by 90 min. Plasma C-peptide levels confirmed that the rise in insulin was of exogenous origin. The glucose infusion rate peaked at 30 min (18.9 ± 3.3 μmol/kg·min) and was almost back to zero by 2 h.
Conclusion: In summary, the clearance and biologic activity of IN-105 are indistinguishable from those of Humulin. IN-105 is reproducibly and rapidly absorbed after esophageal gavage, such that the effect of a 0.25 mg/kg dose on glucose disposal was evident for 2 h. In conclusion, IN-105 moves us one step closer to a biologically effective oral insulin.
Support: Nobex Corporation

PS 70

Glucose measurement and insulin injection devices

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Pump compatibility of insulin aspart compared to insulin lispro with respect to catheter complications and dermal/subcutaneous irritations in type 1 diabetes patients with insulin pump therapy (CSII)

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Background and Aims: Rapidly absorbed insulin analogues, such as insulin Lispro or insulin Aspart have demonstrated similar results considering efficacy and safety in patients with type 1 Diabetes (T1D) undergoing CSII therapy. The purpose of this study was to compare the pump compatibility of these two different insulin preparations with respect to occurrence of catheter complications and dermal/subcutaneous irritations around the catheter insertion sides.
Materials and Methods: In this single-center, randomized, double-blind, 2-period crossover study 20 patients with T1D on CSII therapy were randomized to two 4-week treatment periods on either type of insulin. Each patient received a standardised questionnaire after every 4-week period including 5 categories: 1. pain/burining during bolus administration, 2. inflammation at the insertion side, 3. dermal redness at the insertion side, 4. dermal/subcutaneous indurations and 4. catheter occlusions. Every category was devided into 4 degress of severity leading to the insulin specific side-effect scores (0 points: no complications, 1 point: mild, 2 points: moderate, 3 points: strong) which were then used for statistical analysis. At the end of the study all patients were asked which insulin preparation they would prefer before the order of the insulins was cleared.
Results: Insulin Aspart showed an overall significant ($p<0.005$) lower side-effect score (1.5 ± 1.5 points) than insulin Lispro (7.1 ± 3.6 points). Considering the different categories, insulin Aspart showed significantly less side effects within the categories pain/burning ($p<0.005$), inflammation ($p<0.004$) and dermal redness ($p<0.001$). The categories dermal/subcutaneous indurations ($p=0.188$) and catheter occlusions ($p=0.375$) did not reach statistical significance. From the patients point of view 68.4% would have choosen insulin Aspart, 15.8% would have choosen insulin Lispro, 15.8% had no preference.
Conclusion: Insulin Aspart shows a lower side-effect score considering pump compatibility with respect to occurrence of catheter complications and dermal/subcutaneous irritations compared to insulin Lispro and was overall better tolerated in patients with T1D undergoing CSII therapy. Patients suffering from these complications may benefit from using insulin Aspart.
Support: Novo Nordisk Grant

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Insulin infusion set survival comparing Novolog with Humalog

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Background: There is a common perception amongst our pump patients that infusion sets last longer with more stable diabetes control and less infusion site reactions when Novolog is used rather than Humalog.
Methods: To test this hypothesis, 18 subjects were assigned in a double-blind, cross-over study to use either Novolog or Humalog for 1 week without changing the infusion set. The sequence of insulin use was randomized. Insulin was supplied by the pharmacy in generic bottles labeled insulin "A" or "B". Subjects used a "Silhouette" infusion set, and were asked to continue to use the same set until there was: 1) a blood glucose of > 300 mg/dl that failed to decrease by 50 mg/dl one hour following a correction dose, 2) serum ketones > 0.6 mmol/l associated with a blood glucose > 250 mg/dl, 3) more than 5 mm of redness or firmness at the infusion site 4) or study end (1 week). All subjects wore a Minimed continuous glucose sensor (CGMS) while their study infusion sets were functioning.
Results: The mean (±SD) duration for infusion set survival using Novolog was 4.9 ± 1.8 days and for Humalog was 5.1 ± 1.8 days (p=NS). In each group there were 8 subjects (44%) who used their infusion sets for the full 7 days (6 subjects had both infusion sets last 7 days). Six sets infusing Humalog were removed for hyperglycemia and a failed correction dose (mean duration 3.4 days), and 2 sets infusing Novalog failed for the same reason (mean duration 3.6 days). Three sets infusing Humalog were removed for erythema, induration, and/or pain (mean duration 4.2 days) and 6 sets infusing Novalog were removed for the same reason (mean duration 3.6 days). During the 7 study days, 26% of infusion set sites had erythema >5 mm compared to 8% of CGMS sites, and 10% of infusion sites had induration >5 mm compared to 0% of CGMS sites. Infusion set catheters were stained with dithizone and all catheters had evidence of insulin precipitation in the tubing. Subjects were unable to correctly identify which insulin they were using.
Conclusion: We could not demonstrate a difference between Novolog and Humalog in infusion set survival.
Support: Novo Nordisk

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Insulin treatment satisfaction and fear of self-injection: a comparison of the InnoLet insulin doser and standard vial/syringe

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Background and Aims: InnoLet insulin injection device is specifically designed to overcome practical and psychological obstacles that prevent many patients from effectively managing their insulin treatment. InnoLet is a disposable insulin injection device with a large easy-to-read dial, large push button for injection, and audible clicks for each unit injected. The objective of this study was to evaluate insulin treatment satisfaction, including fear of self-injection, associated with use of the InnoLet insulin doser vs. standard vial/syringe.
Materials and Methods: In a prospective, randomized, open-label, two-period (each 12 weeks), crossover study, 260 patients were enrolled (age ≥18 yrs, with type 1 or 2 diabetes, and receiving NPH or regular or 70/30 insulin for at least 6-months). Patients were excluded if they had a baseline HbA_{1C}>10%, were unable to read/write English, were unable to administer their own injections, were pregnant/lactating, were using antipsychotic medications, or had a history of alcohol abuse or cognitive impairment. Patients were randomized to use either vial/syringe or InnoLet for 12 weeks, and then switched to the alternate treatment for 12 weeks. At the end of each treatment period, patients completed the Insulin Treatment Satisfaction Questionnaire (ITSQ) and Fear of Self-Injection Questionnaire. The ITSQ consisted of 25-items on a 7-point Likert scale which were transformed to six subscales ranging from 0–100; higher scores represented greater satisfaction. The Fear of Self-Injection Questionnaire consisted of 8-items on a 4-point Likert scale; higher scores represented greater fear.
Results: Of the entire cohort, 165 (64%) patients completed the study. Of these, 91 (55%) were in the vial/syringe-to-InnoLet treatment group, 50% were female and mean age was 60 ± 11 years. No significant differences in baseline characteristics were observed in either treatment group. Of the 165 patients, 164 patients completed all six ITSQ subscales and 160 patients completed the entire Fear of Self-Injection Questionnaire in both treatment periods. There was a significant difference in ITSQ scores between the delivery systems. While using the InnoLet system, patients scored higher on all six ITSQ subscales, which included convenience of regimen, lifestyle flexibility, glycemic control, hypoglycemic control, and insulin delivery device satisfaction (Wilcoxon, $p<0.001$). Patients reported significantly lower fear of self-injection after using InnoLet vs. vial/syringe (Mean±SEM: 9.4 ± 0.2 vs. 11.0 ± 0.4; $p<0.0001$).
Conclusion: The InnoLet® may offer greater patient acceptance, improved treatment satisfaction and reduced fear of self-injection. These findings may be clinically significant, given the potential health gains that can be obtained through improved diabetes self-management.
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Which needle length for injecting insulin

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Background and Aims:In the Netherlands most patients with diabetes mellitus that are treated with insulin, use an insulin pen for insulin administration. The injection technique can influence the absorption rate of insulin. Diabetes nurses created transmural guidelines about injecting insulin. It was not clear what would be the correct advice concerning the length of the needles. The aim of this study is to compare the effect of insulin injections using a 5 mm insulin needle with insulin injections using a longer needle,

on HbA_1c, bloodglucose levels, hypoglycaemic events, bleeding, bruising, insulin leakage and pain perception.
Materials and Methods:In this randomised, clinical trial with cross-over design, 52 patients with Diabetes type 1 and 2 were randomised into two groups. Group I started using 5 mm needles for insulin administration, group II continued using their own longer needle. After 13 weeks group I returned to their own longer needle and group II started using the 5-mm needle. During each visit the HbA1c level was measured and a copy was made of the logbook of the patient with the blood glucose measurements. Insulin doses and number of experienced hypoglycaemic events were registered at each visit. The opinion and experiences of the patients regarding the different needles were obtained by using a questionnaire. Within-group analyses were computed, using the Wilcoxon signed Ranks Test. Between-group analyses were computed using the Mann-Whitney U test.
Results: No significant difference was found in HbA1c levels when the longer needle was followed by the 5-mm needle (p=0,63). There is a significant higher HbA1c (p=0,04) when patients return to the longer needle after using the 5 mm needle for three months. However, since only twenty-five patients of group I, were in this situation, this conclusion lacks sufficient power. There were not enough reliable blood glucose measurements to give a valid conclusion about changes in blood glucose levels.
In 86,5% of the cases the 5 mm needle was preferred, in 7,7% the 8 mm needle, 3,8% preferred the 12 mm needle while 1,9% had no preference. The answers given most frequently for choosing the 5-mm needle were that the 5-mm needle was easier, as no skinfold had to be taken and that it caused fewer bruises.
No significant difference was found in insulin leakage (p=0,91) and hypoglycaemic events (p=0,24). The 5-mm needle gave a significant decrease in observed bleeding (p=0,006), bruising (p=0,000) and perceived pain (p=0,003) compared to the longer needle.
Conclusion: As HbA_1c levels did not change, a 5 mm needle can be recommended as a standard advice to patients with a BMI>18 without using a skinfold. Caregivers should translate this standard advice to the individual patient as not all patients preferred the 5-mm needle. Patients should be informed about the existence of various lengths of insulin needles and different firms producing these needles. This study has given no reason to advise short needles to lean patients and longer needles to obese patients but further research is recommended with more patients of the different groups to empower this statement.

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Clinical evaluation of Accu-Chek Aviva, a new system for blood glucose monitoring

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Background and Aims: The new Accu-Chek Aviva blood glucose monitoring system combines easy testing and reliable blood glucose results. It features a measuring range of 10–600 mg/dL, a 5 second test time and the end-dose strip needs only 600 nL of whole blood making it suitable for alternative site testing (AST). Accu-Chek Aviva was evaluated in a multi-site study using whole blood from diabetic patients.
Materials and Methods: Capillary whole blood samples from 95 diabetic patients were tested on five (5) meters with three (3) test strip lots. In addition venous blood samples from 300 diabetic patients were tested on six (6) meters with three (3) test strip lots. EDTA or Li-heparin was used as anticoagulant. The Hexokinase method with deproteinization (Roche Diagnostics on a Hitachi 912) was used to obtain a reference whole blood glucose concentration for each sample. Patient hematocrit was measured using the routine laboratory method. In venous blood samples additionally the concentration of cholesterol, triglycerides, bilirubin, urea, uric acid, and creatinin was measured using the routine laboratory method. Data were analyzed by Passing/Bablok method. Intermediate precision data of three (3) test strip lots were assessed according to the procedure given in ISO 15197.
Results: Glucose concentration ranged from 61-362 mg/dL for capillary blood samples and from 20–395 mg/dL for venous blood samples. Correlation coefficients, intercept, and slope of the Passing/Bablok regression for each sample type and for each center can be found in table 1. Values from the number of meters used at each site were combined. Over a wide range of hematocrit levels (capillary blood 30–50%, venous blood 26–53%) no obvious trend was demonstrated. The in vivo endogenous substances (cholesterol 83–532 mg/dL, triglycerides 39–2576 mg/dL, bilirubin 0.2–22 mg/dL, uric acid 1.6–18 mg/dL, urea 2.4–219 mg/dL, creatinin 0.4–7 mg/dL) did not influence the blood glucose measurement within the range tested. Intermediate precision using ten (10) meters for ten (10) days yielded a standard deviation (SD) of 1.8–2.0 mg/dL for the low control, 3.2–3.9 mg/dL for the normal control, and 6.5–7.7 mg/dL for the high control. The coefficient of variation (CV) ranged from 2.1 to 3.1% for the normal and high controls.
Conclusion: These data indicate that the Accu-Chek Aviva blood glucose monitoring system provides accurate and precise whole blood glucose concentration values for capillary and venous blood.

Table 1

Sample type	Anticoagulant	Site	Lot #	N total	Correlation coefficient (r)	Intercept	Slope
Capillary blood	none	1	072	95	0.993	2.190	0.968
	none	1	073	95	0.992	1.874	0.974
	none	1	074	95	0.990	6.294	0.941
Venous blood	Li-heparin	1	072	100	0.994	–1.677	1.048
	Li-heparin	2	073	100	0.996	–2.555	0.984
	EDTA	3	074	99	0.981	3.613	0.985

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Intensive insulin therapy in the ICU: assessment by interstitial fluid sampling

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Background and Aims: Intensive insulin therapy to keep normoglycaemia reduces mortality and morbidity in critically ill patients. Frequent or continuous glucose monitoring is restricted in critically ill patients due to the high quantities of blood that is required. Hence interstitial fluid (ISF) sampling in the adipose tissue might be an alternative to improve the adjustment of insulin therapy. The primary aim of the study was to investigate whether the glucose concentration in the ISF correlates with plasma glucose. We investigated continuous glucose monitoring in the ISF with a microdialysis based monitoring system.
Materials and Methods: 20 post surgery patients with a glucose level higher than 6.7 mmol/l were investigated in the intensive care unit (ICU) (M/F: 15/5; age 69 ± 7 y, non-diabetics/ type 2 DM/ type 1 DM: 14/6/0; APACHE II score: mean 13, (range 21-6)). Two microdialysis catheters (CMA 60) were inserted into adipose tissue. The dialysate of the first catheter was sampled and analysed for glucose and ions in order to take into account the partial equilibration between the interstitial fluid and the dialysate (ionic reference technique). The second catheter was connected to a continuous glucose monitoring system (SCGM1, Roche Diagnostics). In parallel arterial blood glucose readings were taken hourly. Maximum duration of continuous glucose monitoring was 48 hours initiated after admission at the ICU.
Results: The mean duration of glucose monitoring was 36 ± 15 h. The mean blood glucose value was 7.2 ± 1.4 mmol/l. The mean Pearson correlation coefficient between blood and IFS glucose was $r_{BG\text{-}IFS}$ = 0.812 whereas between blood glucose and the SCGM1 system reading $r_{BG\text{-}SCGM}$ = 0.808 (n.s.). In addition the correlation for different calibration intervals (2-4–6–12–24 hours) of the SCGM1 system was quantified with several evaluation methods (method of residuals, modified error grid analysis (mEGA), predicted error sum of the squares (%PRESS), mean absolute difference (MAD), coefficient of correlation). In general the results improved with shorter calibration intervals. However the calibration procedure is very sensitive to the chosen point of calibration.
Conclusion: There is a significant correlation between blood and ISF glucose in critically ill patients after surgery. Therefore ISF seems to be a promising alternative to blood glucose monitoring in critically ill patients which in addition enables continuous glucose monitoring. This could prove to be clinically useful in the ICU by decreasing workload and providing alarm signals.

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Possible cause of glucose oxidase-based sensor failure during clinical use of continuous glucose monitoring system

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Background and Aims: During clinical use of the continuous glucose monitoring system (CGMS) in some cases the sensor failure occurs. To date there is no applicable explanation for this problem. Studying cases with CGMS disorders, we assume that glucose oxidase (GOD) mixed with bovine serum albumin (BSA) as vital components of the sensor can be affected by various endogenous processes, which may lead to rejection or development of the sensor failure. Our aim was to identify and evaluate discordant tracings due to a sensor error and determine relation to selected patients' characteristics in order to reveal the cause of the sensor failure.
Materials and Methods: Performance data and demographic information were obtained from total of 70 Caucasian patients with diabetes mellitus (DM) selected from our outpatient clinic (47 type 1 DM, 23 type 2 DM, 64% female, 37% GADA positive, mean age±SD 34,6 ± 18,9 years, average duration of DM 6,2 ± 6,6 years, mean HbA_1c =8,6 ± 1,9%, BMI=24,6 ± 5,2). Patients were monitored using the CGMS for 3 or more days during normal activity, in conjunction with self-monitored blood glucose (BG) tests conducted at least 4 times per day. CGMS data were evaluated and classified as satisfactory (A) or impossible to evaluate (B) that were attributed to: insufficient calibration (B1), meter error or insufficient starting sensor electronic signal (ISIG) with no response (B2), sensor error of unknown origin we further investigated (B3). Statistical analysis included correlation, mean absolute difference and paired Student's t test.
Results: 320 glucose profiles from 74 sensors with a mean duration of 111,6 ± 42,24 h were evaluated by comparing sensor data to 1511 capillary BG values (r = 0,84; mean absolute difference=14,8 ± 4,7%). There were total 469,5 h of gaps (5,7%) in continuous glucose monitoring. From12 sensors with serious CGMS disorders 1,7% of gaps were attributed to insufficient calibration (B1). 2 sensors were discarded for technical reasons (B2). During the first 48 h of the continuous glucose monitoring, in 8 subjects (B3; 2,2% of gaps) unexpected ISIG weakening occurred. In 3 cases sensors prematurely lost ability to record glucose values and worked less than 48 h. 5 patients experienced gradual decrease in ISIG leading to error after calibration with steady hyperglycemia, whereas euglycemia did not lead to calibration error despite boundary ISIG value. This type of the sensor failure (B3) strongly correlated with GADA positivity (p=0,00005), other autoimmune disease (p=0,0003), repeated CGMS (p=0,0006) and type 1 DM (p=0,036). No significant correlation was observed with total daily insulin dose (p=0,12), age (p=0,215), HbA_1c (p=0,389), BMI (p=0,798), duration of DM (p=0,983). There were no significant adverse events (for example skin irritation).
Conclusion: This study demonstrated that sensor failure due to an unknown sensor error (B3) is closely correlated to GADA positivity, autoimmune disease, repeated CGMS and type 1 DM. We identified new glucose pattern specific to weakening sensor, which differs from that one of failed for technical reasons. Possible immune response to BSA is under suspicion of the relation to factors that could lead to the eventual sensor failure. The effect of possible agents responsible for GOD degradation on CGMS performance await further study.

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Insulin treatment in type 2 diabetes I

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Pharmacokinetic and pharmacodynamic differences between premixed suspensions of insulin aspart: biphasic insulin aspart 30, 50 and 70

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Background and Aims: Biphasic insulin aspart (BIAsp) 30, 50 and 70 are premixed suspensions of insulin aspart (IAsp), differing in the ratio of soluble to protamine crystallised IAsp. The number denotes the percentage of soluble IAsp. In this randomised, four-period crossover, euglycaemic clamp trial we determined whether the different proportions of IAsp lead to clear differentiation in early pharmacokinetic (PK) and pharmacodynamic (PD) characteristics of the products.
Materials and Methods: Thirty-five healthy subjects (21 men/14 women; age 27 ± 3 years) participated in 4 glucose clamps (clamp level 5 mM, continuous intravenous insulin infusion rate 0.15 mU/(kg × min)) and received a single 0.3 U/kg subcutaneous dose of BIAsp 30, 50, 70, and soluble IAsp on separate days. As IAsp is a clear solution, it was administered open-label, whereas administration of BIAsp 30, 50 and 70 (which are all suspensions) were double-blind. PK and PD assessments were based on serum IAsp concentration and glucose infusion rate (GIR) profiles, respectively. PK and PD endpoints were analysed using a linear mixed model.
Results:
Comparisons of the PK and PD of BIAsp 30, 50, 70 and IAsp

	BIAsp 50 vs BIAsp 30		BIAsp 70 vs BIAsp 50		IAsp vs BIAsp 70	
	Ratio	95% CI	Ratio	95% CI	Ratio	95% CI
Pharmacokinetics						
C_{max}	1.37	[1.18; 1.59]*	1.41	[1.21; 1.64]*	1.34	[1.15; 1.55]*
$AUC_{0-4\,h}$	1.36	[1.23; 1.51]*	1.37	[1.23; 1.52]*	1.22	[1.10; 1.35]*
Pharmacodynamics						
GIR_{max}	1.07	[0.99; 1.15]	1.15	[1.07; 1.24]*	1.08	[1.00; 1.16]*
$AUC_{GIR,\,0-4\,h}$	1.10	[1.03; 1.18]*	1.15	[1.07; 1.23]*	1.01	[0.94; 1.08]

C_{max}: maximum concentration; AUC: area-under-curve, GIR: glucose infusion rate
Ratio and 95% confidence intervals (CI) are presented. * indicates p<0.05

Injection of BIAsp 30, 50, 70, and soluble IAsp resulted in a rapid increase in serum IAsp concentrations, peaking after approx. 60 min for all products. C_{max} and early phase absorption ($AUC_{0-4\,h}$) increased significantly with increasing fractions of soluble IAsp (C_{max} (mU/L): 63 ± 69, 74 ± 22, 108 ± 31, 139 ± 36 and $AUC_{0-4\,h}$ (mU × h/L): 128 ± 49, 168 ± 40, 231 ± 46, 279 ± 50 for BIAsp 30, 50, 70 and IAsp (mean±SD)). The PD results generally reflected the PK results: GIR_{max} and $AUC_{GIR,\,0-4\,h}$ increased with increasing fractions of soluble IAsp. As expected, PD differences were less pronounced than PK differences, however, statistical significance was demonstrated for most comparisons of early PD in this single dose trial.
Conclusion: The PK and PD profiles of BIAsp 30, 50, 70, and soluble IAsp could be clearly distinguished during the early phase after injection, which is dominated by the rapid absorption of soluble IAsp. Differences in the early metabolic effect of the various mixtures should allow for the tailoring of flexible insulin regimens to meet individual prandial insulin needs of patients with diabetes.

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Mean postprandial and 24-hour glucose are lower with insulin lispro mixture 25/75 compared with insulin glargine in patients with type 2 diabetes mellitus

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Background and aims: Insulin lispro mixture 25/75 (25% insulin lispro/75% NPL) (25/75), a treatment including rapid-acting insulin, and insulin glargine (IG), a basal insulin, are each commonly used to treat type 2 diabetes mellitus (T2DM), often in combination with oral antidiabetic agents (OAs). No comparative pharmacodynamic data have been generated for these formulations during the application of a standardized diet over 24 hours.

Materials and methods: We performed a randomized, open-label, crossover comparison of 25/75 administered immediately before morning and evening meals with IG administered at 2200 in 20 patients (pts) with T2DM (10 F, mean age 54 yrs, mean BMI 37 kg/m^2) in an inpatient setting using a standardized diet. Patients were inadequately controlled with insulin and/or OAs at entry (mean HbA_{1c}: 8.4%, range: 7.1–10.9%). Pre-study OAs were continued and pts not on OAs at entry were treated with metformin ≥1500 mg. Both insulins were titrated over 3–4 months to obtain pre-meal blood glucose (BG) <6.0 mM. Patients were then admitted to the research unit for a 24-hr period for measurement of plasma glucose (PG) every 1–2 hrs.
Results: Mean daily insulin doses were 60 units (U) (23 U am and 37 U pm) for 25/75 and 44 U for IG. Fasting PG was not different between the treatments (25/75: 5.8 ± 1.1 vs 5.5 ± 2.1 mM, p=0.67). Mean 2-hr postprandial (pp) PG (all 3 meals combined) and mean 24-hr PG were lower with 25/75 + OA vs IG + OA (9.0 ± 2.0 vs 9.9 ± 1.8 mM, p<0.05; and 6.7 ± 1.0 vs 7.5 ± 1.3 mM, p<0.01). More specifically, 2-hr pp PG was lower after lunch and evening meals (8.7 ± 2.3 vs 9.8 ± 2.9 mM, p<0.01; and 7.9 ± 2.2 vs 9.8 ± 2.0 mM, p<0.01) and at bedtime (4.9 ± 1.4 vs 6.7 ± 1.9 mM, p<0.001). Areas under the glucose curve (AUC) for the entire 24-hr period and areas under the glucose excursion curves (AUC-exc) from 0–4 hrs after meals were also compared. The 24-hr glucose AUC was smaller with 25/75 than with IG (149.4 ± 31.4 vs 175.7 ± 28.9 mM-h, p=0.002). The AUC-exc was significantly lower after the evening meal and tended to be lower after the midday meal with 25/75 than with IG (7.7 ± 5.1 vs 11.2 ± 4.5 mM-h, p=0.006; and 7.6 ± 3.9 vs 9.0 ± 4.7 mM-h, p=0.099). Endpoint HbA_{1c} was lower with 25/75 + OA (6.9 ± 0.5 vs 7.2 ± 0.8%, p<0.05) compared with IG. One episode of mild hypoglycemia (PG <3.5 mM), often asymptomatic, was observed in 8 pts with 25/75 and 3 pts with IG during the 24-hr test period, including 2 pts who had hypoglycemia with both insulins. All hypoglycemia occurred between 2100 and 0400 with the exception of one episode that occurred at 1100 with IG.
Conclusions: In summary, mean 2-hr pp PG, overall 24-hr PG, and HbA_{1c} were lower with insulin lispro mixture 25/75 (a treatment including rapid-acting insulin) + OA than with insulin glargine (a basal insulin) + OA, although the incidence of mild hypoglycemia was higher with 25/75 in the setting of a 24-hr monitoring period. These results support the use of insulin lispro mixture 25/75 + OA in the treatment of pts with T2DM who are not well controlled on OAs and/or insulin.

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Role of tight postprandial glycemia to achieve target HbA_1c levels
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Background and Aims: HbA_1c levels reflect glycemic exposure over the past 2–3 months and are determined by fasting (fPG) and postprandial (ppPG) glucose exposure. Cross sectional studies suggest that attainment of HbA_1c goals requires specific targeting of pp hyperglycemia. We undertook a prospective intervention trial to assess contribution of fPG and ppPG.
Materials and Methods: 164 T2DM (90 male/74 female, HbA1c7.5%) were enrolled in the study. The ultimate goal was to attain HbA1c levels as low as possible without increasing the risk for severe hypoglycemia. Subjects were trained to use self monitoring blood glucose devices and asked to measure a 7 point diurnal blood glucose profile. Before, 90 minutes after each meal and at 11 pm (bedPG) plasma glucose concentrations were determined at baseline and after 3 months of therapy intensification, additionally blood samples for HbA_1c and body weight were obtained.
Results: HbA1c, fPG, ppPG and day long hyperglycemia (mean of prePG, ppPG and bedPG) decreased from 8.7 ± 0.1 to 6.5 ± 0.1%,174 ± 4 to 117 ± 2, 224 ± 9 to 159 ± 3 and 199 ± 4 to 141 ± 2 mg/dl, respectively (all p<0.0001). Weight did not change (84.0 ± 1.4 vs. 82.9 ± 1.5 kg, p=0.36). Hypoglycaemia, (PG <50 mg/dl) was not observed. 73% of patients achieved HbA_1c levels of 7.0%. Both groups were comparable in BMI, initial HbA1c, FPG and ppPG. Those achieving HbA1c of <7.0% had a HbA_1c of 6.20 ± 0.4%, those who did not had a HbA1c of 7.60 ± 0.1%, p<0.001. Despite these differences in HbA1c, FPG did not differ (117 ± 2 vs 119 ± 3 mg/dl, p=0.50). However, those who achieved an HbA_1c <7.0% had significantly lower postprandial and daylong hyperglycemia than those who did not (153 ± 3 vs. 180 ± 5 and 135 ± 2 vs.159 ± 4 mg/dl, respectively both p<0.001).
The greater day-long hyperglycemia was due to greater pp PG which did not return to premeal values with a progressive stepwise increase in plasma glucose levels during the day. As expected, both postprandial and overall glycemia increased stepwise and progressively from the lowest to the highest HbA_1c sixtile. However, the relative contribution of postprandial glycemia decreased progressively from 91 to 43% as HbA_1c levels increased, as assessed by the areas under the curves assuming that the sum of fasting and daylong areas would yield a 100%Conclusion: This study demonstrates for the first time the importance of postprandial glucose excursions to achieve optimal glycemic control, especially when HbA_1c levels of < than 7% are targeted.

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Does treatment schedule impact outcomes? Differences in efficacy and patient reported treatment satisfaction between a twice-daily and a once-daily insulin treatment
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Background and Aims: Prevailing clinical wisdom generally supports the idea that patients prefer fewer daily injections to control their diabetes and that glycaemic control improves with increased number of daily insulin injections**.** This study examined differences in treatment efficacy and patient reported treatment satisfaction with a once-daily insulin injection versus a twice daily injection.
Materials and Methods: Two validated measures of treatment satisfaction were included in a 28 week multicenter randomized controlled treat to target trial with forced titration of 233 insulin naïve type 2 patients comparing once daily injections with insulin glargine and twice daily injections with BIAsp 30 (NovoMix® 30 FlexPen®). Treatment satisfaction was assessed by the Diabetes Treatment Satisfaction Questionnaire (DTSQ) and the Insulin treatment satisfaction Questionnaire (ITSQ). The ITSQ assesses overall satisfaction as well as six components of satisfaction 1) convenience and ease of use, 2) interference with social and daily activities, 3) impact on lifestyle, 4) hypoglycaemic episodes, 5) glycemic control, and 6) insulin delivery system. The DTSQ, developed for diabetes treatments in general, assesses overall satisfaction. Both questionnaires were administered pre-randomization and after 24 weeks of treatment.
Results: More patients on twice daily BIAsp 30 reached target (HbA1c 6.5%) compared to once daily insulin glargine (42% vs. 28%). However, there were no significant differences in overall treatment satisfaction measure between the groups at 24 weeks. There were also no significant differences between treatment regimes for any of the six ITSQ domains. Significant difference was found for some individual items. For example, the twice daily injection regime experienced less pain/physical discomfort (p=0.02) while the once daily group needed less planning for timing of meals (p=0.02).
Conclusion: Conventional clinical wisdom that patients prefer fewer daily injections may not be accurate. This study found no overall differences in treatment satisfaction between a once a day and twice a day injection regimes and that the twice daily treatment allowed more patients to reach HbA1c target goals. Thus, the trade off between satisfaction and efficacy may not be necessary.
Support: Novo Nordisk A/S

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Insulin detemir incurs a lower risk of hypoglycaemia than NPH insulin for any level of HbA_{1c} when added to oral agents in a treat-to-target protocol for patients with type 2 diabetes
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Background and Aims: Improved glycaemic control through the use of insulin has been shown to reduce the incidence and delay the progression of the long-term complications of type 2 diabetes. However, improved glycaemic control also increases the risk of hypoglycaemia and the inverse correlation that exists between hypoglycaemic frequency and HbA_{1c} can, in clinical practice, limit the achievable level of glycaemic control through impaired tolerability. In comparative clinical trials against NPH insulin in type 1 and type 2 diabetes, the basal insulin analogue insulin detemir has been associated with reduced within-subject variability in pharmacodynamic effect, less weight gain and a reduced risk of nocturnal hypoglycaemia. In most cases, these insulins were compared in the context of basal-bolus therapy. In the present study, the relationship between hypoglycaemia and HbA_{1c} was compared when twice-daily insulin detemir or NPH insulin were added to current oral antidiabetic drugs (OADs) in type 2 diabetes.
Materials and Methods: The 26-week multicentre, randomised, parallel group study included 476 insulin-naive patients with type 2 diabetes inade-

quately controlled on OADs. A goal-directed titration algorithm was used to achieve as good glycaemic control as possible within a 24-week active treatment period. Targets for pre-breakfast and pre-dinner plasma glucose were ≤6 mmol/L based on home-measured plasma glucose levels. Relative risk for hypoglycaemia was estimated using a negative-binomial distribution with a log-link function, and plotted against HbA_{1c}.
Results: Mean HbA_{1c} was decreased by 1.84% and 1.90% points with insulin detemir and NPH insulin, respectively, to endpoint values of 6.58% and 6.46% (ns) with sufficient spread of values to model the relationships of HbA_{1c} to risk of hypoglycaemia. This analysis indicated that the overall relative risk for hypoglycaemia was 39% lower for insulin detemir than for NPH insulin (0.61, p=0.008), with a risk reduction favouring insulin detemir observed at every level of HbA_{1c} (table). Moreover, the plotted curves of hypoglycaemic risk against HbA_{1c} indicated that the extent of this risk reduction progressively increased with improving control.
Conclusion: These findings suggest that for patients with type 2 diabetes inadequately controlled by OADs, the addition of insulin detemir in preference to NPH insulin is likely to result in the achievement of lower levels of HbA_{1c} for any tolerable frequency of hypoglycaemia.

	HbA1c	6.0%	7.0%	8.0%	9.0%
Hypoglycaemic events per patient year	NPH	10	6	4	2
Hypoglycaemic events per patient year	Detemir	6	4	2	1

Support: Novo Nordisk

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Introduction of insulin glargine to basal-bolus therapy improves metabolic control in patients with type 2 diabetes in everyday clinical practice
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Background and Aims: The aim of this study was to observe the effect of insulin glargine as the basal insulin in basal-bolus therapy for patients with inadequately controlled type 2 diabetes in everyday practice.
Materials and Methods: In this 6-week, uncontrolled, observational study, 5696 patients with type 2 diabetes poorly controlled on basal-bolus insulin therapy (basal: 98.6% NPH insulin; 1.4% lente insulin; bolus: 58.4% human insulin; 16.2% insulin lispro; 16.0% insulin aspart; 9.5% other) received insulin glargine (median time of application 21:00 hours) treatment in combination with rapid-acting or regular human insulin. Dosing decisions were made at the physicians' discretion. Mean±standard deviation (SD) target fasting blood glucose (FBG) and HbA_{1c} levels set by the physicians were 6.7 ± 1.3 mmol/L and 6.8 ± 0.6%, respectively, after 6 weeks of treatment. Data relating to changes in HbA_{1c}, FBG, postprandial blood glucose (BG) and insulin dose are presented here.
Results: At baseline, mean±SD age was 61.2 ± 10.2 years, the average duration of previous basal-bolus treatment was 1.6 ± 2.6 years, mean±SD HbA_{1c} was 8.2 ± 1.1%, mean±SD FBG was 9.5 ± 2.2 mmol/L and mean±SD body mass index was 30.0 ± 5.0 kg/m^2. After 6 weeks of insulin glargine therapy, patients achieved target FBG levels, postprandial BG levels improved and the insulin glargine dose increased (Table). The mean±SD bolus insulin dose was 40.3 ± 21.1 IU (9.1 ± 11.1 IU/bread exchange unit) at baseline and decreased to 30.5 ± 18.0 IU (9.3 ± 11.7 IU/bread exchange unit) after 6 weeks. A total of 40 adverse drug reactions were reported in 19 patients, 20 of which were hypoglycaemic events.

	FBG (mmol/L)	HbA_{1c} (%)	2-hour postprandial BG (mmol/L)	Insulin glargine dose (IU)
Baseline	9.5 ± 2.2	8.2 ± 1.1	10.3 ± 2.4	22.9 ± 11. 0*
6 weeks	6.8 ± 1.4	7.3 ± 0.8	7.8 ± 1.6	27.3 ± 12.9

*Mean starting dose of insulin glargine

Conclusion: These results are consistent with data obtained in clinical trials and suggest that in everyday practice, patients with type 2 diabetes poorly controlled on basal-bolus regimens achieve better glycaemic control when switching to insulin glargine as the basal insulin, in order to achieve target glycaemic control.
This study is supported by sanofi-aventis.

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Treatment preference in patients with type 2 diabetes poorly controlled on oral antidiabetic drugs - a cross-sectional non-interventional survey
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Background and Aims: Patients with type 2 diabetes (T2D) failing oral antidiabetic treatment (OADs) often refuse to move onto subcutaneous (SC) insulin for many years. The aims of this survey were to: (1) inform the patients about the necessity for treatment optimization; (2) discuss the different treatment options; (3) define future treatment; (4) describe, using lab parameters, the current risk profile regarding micro- and macrovascular diabetes-related diseases.
Materials and Methods: This cross-sectional survey included 239 T2D patients poorly controlled on OADs. The patients discussed treatment optimization options with a physician. In Step One, patients had the choice to optimize treatment by using an additional OAD or to switch to SC insulin. Patients who opted for OAD optimization were included in Step Two. After another discussion regarding treatment optimization the patients had the choice between OAD, SC insulin, or inhaled insulin (INH; Exubera®). All final decisions were documented. Blood was taken to investigate the actual status of glycemic control and to obtain information regarding cardiovascular (CV) risk factors. The statistical analysis was performed descriptively.
Results: 207 blood samples were available for analysis; treatment preference was documented in 239 patients. In Step One, 80% of the patients decided to continue on OAD and 20% chose SC insulin. 191 patients entered Step Two. Only 4% opted for SC insulin, 41% opted for INH, and 55% decided to stay on OAD. The mean lab values were as follows: HbA_{1c} 8.7 ± 1.4%; LDL-cholesterol 131 ± 36 mg/dl; intact proinsulin 22.1 ± 17.8 pmol/l (elevated in 64% of the patients) and adiponectin 6.4 ± 4.6 µg/dl (elevated in 70% of the patients). Measurements of high-sensitivity C-reactive protein (hsCRP) indicating moderate to severe CV risk was present in 64% of the patients. Based on the HbA_{1c} values and the current treatment, treatment optimization was necessary and recommended in all patients.
Conclusion: The entire study population required treatment optimization. While acceptance of insulin is low when the only option is SC, the willingness to switch to insulin can be increased dramatically if INH is added as a treatment option. INH may help overcome the barriers to insulin treatment in a subset of patients with T2D. Further investigation is required to understand the barriers that appear to limit the initiation of insulin treatment. In addition, other factors that influence decision-making towards the different treatment options require research.

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Impact of Ramadan fasting on some anthropometric and biochemical parameters in Bangladeshi type 2 diabetic subjects
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Background and Aims: Fasting during one month of Ramadan is one of the most important religious practices of millions of Muslims. However, studies related to metabolic changes in Ramadan are very few in number. The aim of the study was to explore the effect of Ramadan fasting on certain anthropometric and biochemical parameters, and on energy expenditure of type 2 diabetic subjects.
Materials and Methods: Ninety two type 2 diabetic subjects (male: female ratio 61:31, age 47 ± 9 years, mean±SD) were selected from the Out Patient Department of the tertiary care hospital of Diabetic Association of Bangladesh. Data were collected in a pre-designed interviewer administered questionnaire. The first survey was taken 1-4 weeks before Ramadan and the second survey was done during 3–4 weeks of Ramadan. The total daily energy expenditure of the subjects was calculated by a factorial method using Physical Activity Ratio (PAR). Dofference between after Ramadan and before Ramadan values were calcutated using paired t- test.
Results: There was no significant difference between before Ramadan and during Ramadan values of BMI and waist-to-hip ratio. However, during Ramadan fasting serum glucose values (5 ± 1.2 mmol/dl mean±SD), fructosamine (145 ± 32 µmol/l), total cholesterol (208 ± 36 mg/dl) and creatinine (1.1 ± 0.18 mg/dl) were significantly higher compared to before Ramadan values (4.7 ± 0.83, 128 ± 38, 197 ± 37, 1.1 ± 0.21 respectively; p=0.0001 for FPG, p=0.001 for fructosamine, p=0.005 for total cholesterol and p=0.01 to creatinine). The median (range) duration of exercise among

all subjects was 45(0–180) min/day. Before Ramadan the duration of physical exercise was adequate in 30% subjects, but during Ramadan the corresponding value decreased to only 8%. Duration of exercise was significantly lower during Ramadan (9.8 ± 17.4 min/day) compared to before Ramadan value (44.2 ± 35.8 min/day, p=0.0001). Total energy expenditure of the subjects was significantly lower during Ramadan (2178.2 ± 215.9) compared to before Ramadan value (2232.8 ± 209.7, p=0.0001).
Conclusion: Ramadan fasting is associated with a deteriortion of metabolic control in diabetic patients and this is related, at least partly, to decreased energy expediture as a consequence of reduced physical activity. Coordinated education and motivation program should be undertaken to improve awareness on this issue.

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Insulin treatment in type 2 diabetes II

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Patients with type 2 diabetes not achieving glycemic targets on OADs with/without basal insulin can reach HbA_{1c} targets with biphasic insulin aspart 30/70

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Background and Aims: The purpose of this study was to demonstrate that biphasic insulin aspart 30/70 (BIAsp 30, NovoMix® 30), which provides both basal and bolus insulin coverage, can reduce HbA_{1c} levels to ≤6.5%. Guidelines for initiating and titrating insulin treatment with BIAsp 30 are provided.
Material and Methods: In this 48-week, multi-center, open-label trial, patients with T2DM, not achieving glycemic targets on OADs (with or without once-daily basal insulin therapy with NPH or glargine) were titrated with BIAsp 30 to target plasma glucose (PG) levels by the algorithm below. In Phase 1, patients initiated treatment with 12U BIAsp 30 once-daily before supper and titrated the dose based on fasting PG values. Dosing frequency was increased to twice daily in Phase 2 (pre-supper + pre-breakfast), and to three times daily in Phase 3 (pre-supper + pre-breakfast + pre-lunch) at 16 and 32 weeks, respectively, based on HbA_{1c} levels. The breakfast dose was initiated with 3U BIAsp 30 if FBG <6.1 or with 6U BIAsp 30 if FPG >6.1; the lunch dose was initiated with 3U BIAsp 30. Patients attaining an HbA_{1c} value ≤6.5% at the end of any phase were considered to have completed the study. BIAsp 30 was administered within 15 minutes before the meal. Pre-breakfast insulin was adjusted based on pre-supper PG values; pre-lunch insulin, on 2-hour post-lunch PG values.

Plasma glucose (mmol/L)	<4.4	4.4 to 6.1	6.2 to 7.8	7.83 to 10	>10
Pre-breakfast or supper dose change (U)	-3	No change	+3	+6	+9
Plasma glucose after lunch (mmol/L)		<5.6	5.6 to 7.8	7.83 to 10	>10
Lunchtime dose change (U)		-3	No change	+3	+6

Results: Patients entering the study had mean HbA_{1c} of 8.6%± 0.8 and age of 56.7 ± 11.5 yrs. The numbers of patients reaching HbA_{1c} targets after each phase are summarized below. Mean daily insulin doses for subjects who achieved an HbA_{1c} ≤6.5% at the end of Phases 1, 2, and 3 were 0.6, 0.9, and 1.1 U/kg, respectively.

	Phase 1	Phase 2	Phase 3	Total
Subjects Enrolled	100	68	25	100
Subjects Discontinued	11*	15*	0	26
Subjects attaining HbA_{1C} ≤6.5% [N (%)]	21 (21)	29# (43)	8 (32)	58 (58)
Subjects attaining HbA_{1C} <7.0% [N (%)]	41 (41)	47 (69)	16 (64)	78 (78)

*One subject in Phase 1 and 10 subjects in Phase 2 had HbA_{1c}<7.0% when they discontinued.
#One subject in Phase 2 had HbA_{1c}≤6.5% when he discontinued.

Conclusion: BIAsp 30, when progressively titrated, enables a high percentage of patients to achieve glycemic targets when added to OAD therapy in T2DM unsuccessfully treated with OADs and basal insulin only.
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Clinical pharmacological aspects of biphasic insulin aspart 50 and biphasic insulin aspart 70 in non-obese and obese patients with type 2 diabetes

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Background and Aims: Biphasic insulin analogs (premixed fast-acting and intermediate-acting) have been developed to improve overall glycemic control in diabetic patients. The aim of the present study was to compare the pharmacodynamics of thrice daily injections of biphasic insulin aspart 50 ((BIAsp50), 50% fast-acting insulin aspart) versus biphasic insulin aspart 70 ((BIAsp70), 70% fast-acting insulin aspart) in sub-groups of non-obese and obese patients with type 2 diabetes.
Materials and Methods: A total of 72 type 2 diabetic patients, 38 non-obese (Body Mass Index (BMI) 23–28 Kg/m^2) and 34 obese (BMI 30–35 Kg/m^2), with stable glycemic control on any insulin regimen (HbA_{1c} ≤9.0%) were included in this stratified (by BMI), double-blind, randomized cross-over study. In two periods of 4 weeks each the patients were treated thrice daily with BIAsp 50 or BIAsp 70 in random order. During both periods, insulin doses were titrated individually. At the end of each period 24-hour serum profiles of glucose, insulin aspart and C-peptide were recorded. Patients administered insulin before receiving standardized meals at breakfast, lunch and dinner on the profile days. The primary endpoint was the area under the serum glucose concentration curve ($AUC_{Glu(0–24\,h)}$).
Results: Comparison of BIAsp 50 and BIAsp 70 treatment revealed no statistically significant difference in overall glycemic control for non-obese or obese type 2 diabetic patients: $AUC_{Glu(0–24\,h)}$ BIAsp 50/BIAsp 70 ratio for non-obese was 0.97 (95% CI: 0.90–1.05), and $AUC_{Glu(0–24\,h)}$ BIAsp 50/BIAsp 70 ratio for obese was 0.98 (95% CI: 0.92–1.05). In general, fasting serum glucose (FSG) was high, and treatment with BIAsp 70 produced statistically significant higher FSG levels than BIAsp 50 in both BMI groups: FSG BIAsp 50/BIAsp 70 ratio for non-obese was 0.90 (95% CI: 0.84–0.96), P=0.0020, and FSG BIAsp 50/BIAsp 70 ratio for obese was 0.90 (95% CI: 0.84–0.97), P=0.0055. With both treatments and in both BMI groups, the majority of hypoglycemic episodes occurred during daytime, the frequency being highest for the non-obese group.
Conclusion: There was no difference in overall glycemic control after thrice daily BIAsp 50 versus thrice daily BIAsp 70 in non-obese and obese patients with type 2 diabetes, respectively. In general, FSG was high, and in both BMI groups significantly higher with BIAsp 70 compared with BIAsp 50, suggesting that a higher proportion of intermediate-acting insulin injection such as BIAsp 30 is needed for night-time glycemic control. Data indicates that the choice between the two treatments should be considered on an individual basis. No safety concerns were raised by this study.

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Metabolic control and cost-effectiveness of prandial insulin therapy for patients with type 2 diabetes - results of "PHAZIT"

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Background and Aims: Providing short-acting insulin at mealtimes is an increasingly common therapy for patients with T2DM despite a lack of information regarding the cost-effectiveness of prandial use of insulin. The PHAZIT study has been designed to compare the prandial use of a short-acting insulin analogue (insulin aspart; ASP) with human short-acting insulin (HI) – both in combination with Metformin (MET) – with regard to metabolic control, weight and incurred treatment costs.
Materials and Methods: PHAZIT is a prospective, non-randomized observational study of patients with T2DM from 53 German outpatient diabetic centres. Only patients with insufficient metabolic control (HbA1c >7.0% and <12.0%) under combination therapy with OHA (incl. MET) were included in the study. At baseline these patients were switched to a combination of ASP/MET or HI/MET. Implemented quality standards followed the national guidelines for health economic evaluation, a central laboratory (HbA_1c) and regular monitoring. The primary outcome parameter was change of HbA_1c after 24 weeks of therapy compared to baseline. Secondary parameters were insulin dosage, change of weight and treatment costs. Clinical and economic data were analysed only for the per protocol (PP) population. The external validity of documented diagnoses, medical findings and treatment costs was verified by t-test in comparison to TEMPO-study.
Results: 490 patients (ASP 250; HI 240) were analysed in the PP-population. Patients in both groups were comparable regarding age, duration of diabetes, gender and known co-morbidities such as hypertension and dyslipidaemia. Starting from identical baseline HbA1c (8.7%) a significant improvement in metabolic control was observed in both groups (Tab. 1). The change in HbA1c was achieved in patients from the ASP/MET group with 15% less daily insulin dosage than for patients in the HI/MET group (p<.001). Moderate weight loss was observed in the ASP/MET group while there was weight gain in the HI/MET group (p<.05). There was no significant difference in total outpatient costs and in cost-effectiveness ratio, defined as outpatient costs per HbA1c-reduction of 0.5%. The t-tests between PHAZIT and comparative trails like TEMPO-study showed no substantial difference between study populations concerning morbidity, findings and costs (.108<p<.828).
Conclusion: Prandial insulin therapy in combination with MET proved to be a very potent therapy option for T2DM to improve metabolic control. The use of ASP was as cost-effective as the use of HI. Additional benefits of ASP like weight loss improve quality of life. The analysis of the external validity confirmed the study results to be transferable into daily practice.

Table 1

Parameter (mean +/– standard deviation); [95%CI]; **p<0.01; *p<0.05	ASP/MET (n=250)	HI/MET (n=240)
Change in HbA1c (%-points)	–1.7 (+/–1.37) [–1.9; –1.5]	–1.7 (+/–1.26) [–1.8; –1.5]
Bodyweight (kg at start of treatment)	90.1 (+/–18.9)	89.8 (+/–17.7)
Change of weight (kg)	–0.37* (+/–4.42) [–0.94; +0.19]	+0.66* (+/–4.63) [+0.08; +1.23]
Daily dose of metformin (mg after 24 weeks)	1552 (+/–571)	1546 (+/–564)
Daily dose of insulin (Units at start of treatment)	23.4** (+/–12.6)	27.3** (+/–14.8)
Daily dose of insulin (Units after 24 weeks)	29.4** (+/–16.0)	35.8** (+/–16.0)
Physician's fee for outpatient treatment (EURO)	198.76 (167.3)	209.42 (136.6)
Means for cure and auxiliary (incl. test strips) (EURO)	390.57 (300.8)	415.24 (257.4)
Medication (EURO)	501.91** (290.4)	424.23** (299.4)
Total outpatient costs (EURO)	1091.25 (531.0)	1048.89 (460.2)
Outpatient costs (EURO) per HbA1c-reduction of 0.5%	320.65	314.75

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Insulin glargine improves glycaemic control in patients with type 2 diabetes on multiple daily insulin injections confirmed by using the CGMS

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Background and Aims: Multiple daily injection therapy (MDI) is common in patients with type 2 diabetes who require insulin.
Materials and Methods: This multicentre, open, single-arm study assessed glycaemic control in 479 patients with type 2 diabetes treated on their previous MDI regimen of NPH insulin plus mealtime insulin for 18.8 days, followed by 59.5 days' treatment with insulin glargine plus mealtime insulin. Blood glucose was measured using a continuous glucose monitoring system (CGMS). 72-hour CGMS registration was performed at the end of the NPH insulin (baseline) and insulin glargine period (endpoint). The area under the curve (AUC), representing the extent patients were within, above or below defined blood glucose ranges detected by CGMS averaged to 24 hours, was calculated.
Results: The full analysis set consisted of 367 patients (mean age 59.2 years; duration of diabetes 12.7 years; body mass index [BMI] 31.7 kg/m^2). All data are presented as baseline (NPH insulin) versus endpoint (insulin glargine), except HbA_{1c}, which was measured at Visit 1 (Week 0) and Visit 9 (Week 10). After switching to insulin glargine, mean HbA_{1c} dropped from

6.90 to 6.67% (p <0.001) and mean baseline fasting blood glucose decreased from 7.8 mmol/L (140 mg/dL) to 7.2 mmol/L (129 mg/dL); p <0.001. Compared to NPH insulin, AUC within the target blood glucose range of 3.9–7.5 mmol/L [70–135 mg/dL] was significantly increased with insulin glargine (428.7 vs 461.4 hr.mg/dL, p <0.001). In addition, AUC showing blood glucose levels ≥7.5 mmol/L (≥135 mg/dL) (552.4 vs 445.4 hr.mg/dL) or ≥10 mmol/L (≥180 mg/dL) (182.9 vs 135.8 hr.mg/dL) decreased significantly with insulin glargine (p <0.001). AUC with blood glucose levels ≤3.3 mmol/L (≤60 mg/dL) during the insulin glargine period showed no clinically or statistically significant change (4.4 vs 5.4 hr.mg/dL; p=0.255), although symptomatic hypoglycaemia reported by patients dropped from baseline to endpoint during CGMS registration (71 [19.3%] to 59 patients [16.1%]).
Conclusion: Switching MDI-treated patients with type 2 diabetes from NPH insulin to insulin glargine improves glycaemic control as confirmed by CGMS readings. The blood glucose of the insulin glargine-treated patients remained within the preferred blood glucose ranges more frequently, as shown by AUC data, and patients experienced less symptomatic hypoglycaemia.
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The efficacy of long-term insulin glargine and oral antidiabetic therapy in patients with type 2 diabetes with different body mass index
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Background and Aims: Achieving and maintaining target HbA_{1c} (<7%) in patients with type 2 diabetes reduces the risk of late diabetic complications. To date, few data exist regarding the long-term efficacy of adding insulin therapy to oral antidiabetic agent (OAD) therapy in maintaining glycaemic control. Previous results of a 9-month, uncontrolled observation study (n=12,216) demonstrate that the addition of insulin glargine, a basal insulin analog, to existing OAD therapy is associated with reductions in HbA_{1c} to target levels. This analysis aimed to further establish the effects of long-term once-daily insulin glargine and OAD therapy on glycaemic control in patients with Type 2 diabetes in a 20-month extension of this uncontrolled observational study. After 9 months of observation, an extension of up to 20 months was offered to the participating physicians.
Materials and Methods: 20-month data were available for 2721 patients; the composition of the patient groups is shown in the table. Dosing decisions, including changes to OAD therapy, were made at the physicians discretion, reflecting everyday practice. Analyses according to body mass index (BMI) were performed after 20 months to determine changes in HbA_{1c} and fasting blood glucose (FBG).
Results: At baseline, mean±standard deviation age was 63.8 ± 11.3 years, HbA_{1c} was 8.6 ± 1.4%, FBG was 11.2 ± 3.1 mmol/L and BMI was 29.0 ± 4.6 kg/m^2. Reductions in HbA_{1c} and FBG were observed after 9 months' treatment with insulin glargine plus OADs and these reductions were maintained after 20 months of treatment (Table) in all treatment groups, irrespective of BMI. Only three hypoglycaemic episodes were reported in the overall population.

	HbA_{1c} (%)	FBG (mmol/L)	BMI (kg/m^2)	Dosage (IU)
All patients				
Baseline	8.6 ± 1.4	11.2 ± 3.1	29.0 ± 4.6	24.2 ± 7.2
9 months	7.1 ± 1.0	7.2 ± 1.8	28.6 ± 4.7	20.2 ± 9.5
20 months	7.0 ± 1.0	7.3 ± 2.1	28.7 ± 4.7	22.3 ± 9.9
Group 1: (n=432)				
BMI <19 and <25 kg/m^2				
Baseline	8.4 ± 1.4	11.0 ± 3.1	23.5 ± 1.3	13.0 ± 6.3
9 months	6.9 ± 0.9	7.0 ± 2.0	24.3 ± 2.2	18.6 ± 8.1
20 months	6.9 ± 0.9	6.9 ± 1.9	24.6 ± 2.5	20.5 ± 9.3
Group 2: (n=1184)				
BMI ≥25 and <30 kg/m^2				
Baseline	8.6 ± 1.4	11.0 ± 2.9	27.5 ± 1.4	13.9 ± 6.5
9 months	6.9 ± 0.9	7.1 ± 1.6	27.3 ± 2.5	19.3 ± 9.1
20 months	6.9 ± 1.0	7.2 ± 1.9	27.4 ± 2.7	21.6 ± 9.0
Group 3: (n=869)				
BMI ≥30 kg/m^2				
Baseline	8.8 ± 1.4	11.5 ± 3.3	34.0 ± 3.8	15.3 ± 8.1
9 months	7.1 ± 1.0	7.4 ± 1.9	32.6 ± 4.7	22.1 ± 9.9
20 months	7.1 ± 1.0	7.5 ± 2.1	32.4 ± 5.0	23.9 ± 10.4

Conclusion: These data suggest that, in daily practice, the introduction of insulin glargine to continued OAD therapy can facilitate both the attainment and maintenance of target HbA_{1c} in patients with type 2 diabetes, irrespective of BMI.
This study was supported by sanofi-aventis.

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Insulin detemir is associated with less weight gain than NPH insulin, especially in the very obese, when used in basal bolus therapy for patients with type 2 diabetes
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Background and Aims: Weight gain can be an undesirable side effect of insulin treatment. Previous clinical trials have shown consistently less weight gain with the basal insulin analogue insulin detemir than with NPH insulin. This analysis sought to ascertain whether the weight advantage of insulin detemir is related to a patient's initial body mass index (BMI).
Materials and Methods: Data were pooled from two randomised, parallel-group multicentre trials (one of 22 weeks, one of 24 weeks) in which previously insulin-treated patients with type 2 diabetes (n=900) had their regimen intensified to basal-bolus therapy. Patients were aged at least 18 years, had an HbA_{1c} less than 12.0%, and a history of diabetes of at least 12 months. They had been on insulin (basal-bolus regimen, biphasic insulin or oral hypoglycaemic agents in combination with insulin) for at least 2 months. Patients received once- or twice-daily insulin detemir (with mealtime insulin aspart) or NPH insulin (with mealtime insulin aspart or human soluble insulin). Weight change from baseline was analysed as a function of baseline BMI, with the statistical model including treatment (NPH or insulin detemir), BMI at entry (as a covariate), trial identification, interactions between treatment and trial identification and treatment and BMI. Weight change data were also stratified by four categories of baseline BMI.
Results: Overall, NPH insulin-treated patients gained significantly more weight (1.2410 kg) than insulin detemir-treated patients (0.4199 kg, estimated difference -0.8210, p=0.0005,). For insulin detemir the estimated slope for the linear regression of change in body weight by entry BMI was negative (-0.03264), but non-significantly different to zero. For NPH insulin the estimated slope was significantly positive (0.07481, p=0.025) indicating a tendency for increased weight gain with increasing BMI. A between-treatment difference in weight gain was most marked in very obese patients (BMI>35). In this subgroup, patients lost weight during treatment with insulin detemir, whereas NPH-treated patients had the largest weight gain (table).
Conclusion: Insulin detemir used as the basal component of intensified insulin therapy for type 2 diabetes is associated with less weight gain than NPH insulin. This potential clinical advantage may be especially marked in the treatment of very obese patients.

Baseline BMI	Insulin detemir N	Insulin detemir Weight gain (kg)	NPH insulin N	NPH insulin Weight gain (kg)
≤25	74	0.41	61	1.31
>25–30	219	0.65	145	0.80
>30–35	159	0.47	101	1.25
>35	73	–0.45	52	2.40

Support: Novo Nordisk

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Substitution of first phase insulin response with insulin aspart in patients with newly onset type 2 diabetes
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Background and Aims: Loss of first phase insulin response (AIR) is an early and characteristic deficiency in patients with type 2 diabetes. We have previously shown that the impairment of AIR is correlated to increased postprandial glucose increment. The aim of the present study was to assess substitution of deficient first phase insulin response with the rapid acting insulin analogue, insulin Aspart, on postprandial blood glucose increment in patients with diet treated type 2 diabetes. Fur-

thermore, the optimal dose and timing of insulin injection in relation to a meal was evaluated.
Materials and Methods: Twenty patients with newly diagnosed Type 2 diabetes, mean age 61,5 ± 12,4 years, diabetes duration 1,0 ± 1,8 years, BMI 29,1 ± 7,9 kg/m^2 and HbA1c 7,3 ± 0,9% participated. All patients were tested negative for first phase insulin response. In a randomised double blind, double dummy design patients were examined at four meal tests with injection of insulin Aspart and/or placebo 30 and 15 minutes before the meal. Following insulin regimens were used: (injection 1 at -30 minutes/injection 2 at -15 minutes): a) Placebo / Placebo b) insulin Aspart 0,04 IU/kg BW / Placebo, c) Placebo / insulin Aspart 0,04 IU/kg BW and d) insulin Aspart 0,08 IU/kg BW / Placebo. The meal was a standard breakfast containing 2099 KJ as 69g carbohydrates, 12g fat and 21g protein. Postprandial blood glucose increment was defined as the incremental area under the plasma glucose curve 240 minutes after the meal ($AUC_{glu\ -30–240\ min}$). The difference between baseline blood glucose, t =-30 min, and maximal blood glucose after meal (C_{max}) were expressed as $\Delta C_{max.}$
Results: The postprandial increment in blood glucose expressed as $AUC_{glu\ -30–240\ min}$ was significantly reduced with all insulin regimens b) 104,7 ± 63,4 mmol/l × min, c) 121,6 ± 67,2 mmol/l × min, d) –83,9 ± 86,9 mmol/l x min compared to placebo (394,7 ± 80 mmol/l) with insulin regimen d) significantly more effective as compared to b) and c). The maximal postprandial blood glucose increment ΔC_{max}, was significantly lower for all three insulin regimens as compared to placebo: a) 4,6 ± 0,4 mmol/l, b) 2,1 ± 0,5 mmol/l, c) 3,0 ± 0,3 mmol/l and d) 2,1 ± 0,4 mmol/l, p<0,002. One patient developed hypoglycaemia after injection of 0,08 IU and the test was interrupted 155 minutes after the meal (at a plasma glucose of 2,8 mmol/l and subjective symptoms of hypoglycaemia). No patients developed hypoglycaemia after injection of 0,04 IU/kg BW insulin Aspart or placebo.
Conclusion: Injection of small doses of insulin Aspart (0,04–0,08 IU/kg BW) given to diet treated early type 2 diabetes patients 30 minutes before a meal significantly reduces the postprandial blood glucose increment to levels seen in non-diabetic persons.

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Comparison of the pharmacodynamics and pharmacokinetics of bolus subcutaneous insulin glulisine and insulin lispro in obese patients with type 2 diabetes

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Background and Aims: To compare the pharmaco-dynamics and -kinetics of bolus subcutaneous insulin glulisine and insulin lispro in obese subjects with type 2 diabetes injected immediately before 3 standardised meals during a 12 hour study day.
Materials and Methods: A randomized, open-label, two-arm, cross-over study design was employed in 18 obese subjects with type 2 diabetes treated with oral hypoglycaemic agents (OHAs). Following an overnight fast subjects received subcutaneous (anterior abdominal wall) insulin glulisine or insulin lispro (0.15 IU/kg) at 4 h intervals immediately prior to 3 standardised 500 kcal test meals (breakfast, lunch and dinner). OHAs were omitted on study days. After one week the same procedure was repeated using the alternative insulin preparation. Frequent blood samples were taken throughout the study days for measurement of glucose, insulin and C-peptide concentrations. Plasma concentrations of insulin glulisine and insulin lispro were measured using specific assays with no cross reactivity with human insulin.
Results: Subjects were aged (mean) 60 (range 41–71) years, BMI 37.4 (33.2–46.5) kg/m^2, HbA1c 7.8 (6.0–10.9) %. Following the meals no differences in the plasma glucose day profiles were observed; AUC0–240 min (mmol/L · min)186.3 vs 194.7 (p=0.71), AUC240–480 min 94.9 vs 113.1 (p=0.43), AUC480–720 min 375.6 vs 365.1 (p=0.65) during the insulin glulisine or lispro study days, respectively. Maximum plasma glucose excursion was greater following lunch during the insulin lispro study day 3.39 vs 3.72 (p=0.26), 2.58 vs 3.44 (p<0.01), 5.11. vs 5.20 (p=0.83) mmol/l. The overall difference in maximum plasma glucose excursion between glulisine and lispro was statistically significant (p=0.007) with the glucose excursion following administration of insulin glulisine being smaller than that following administration of insulin lispro by 12%. Geometric mean insulin analogue concentrations were consistently higher following administration of glulisine; AUC0–240 min (uU/mL · min) 14837 vs 12488 (p<0.01), AUC240–480 min 23850 vs 17293 (p<0.01), AUC480–720 min 25767 vs 18858 (p<0.01). For both plasma glucose and insulin there were statistically significant differences between the 3 meals, with higher values observed post breakfast.
Conclusion: In this group of obese subjects with type 2 diabetes the two insulins showed bioequivalence on all outcomes analysed, other than the maximal glucose excursion which was less following glulisine administration. There was however, a difference in the pharmacokinetics of the two insulin analogues, with plasma insulin levels higher with insulin glulisine. The results of this study may have implications with respect to the beta-cell sparing effects of short acting insulins in addition to the timing of the introduction and administration of prandial insulin.
Support: Aventis

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Insulin treatment in type 2 diabetes III

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A clinical trial of continuous subcutaneous insulin infusion versus multiple daily injections in older adults with type 2 diabetes

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Background: In the middle-aged adults with type 2 diabetes mellitus (T2DM), intensive glycemic management can delay or prevent the development of microvascular and neuropathic complications.While lifestyle changes and oral antidiabetic medications can improve glycemic control early in the course of T2DM, insulin is often required to achiefve goals later in the course of the disease.The efficacy and safety of alternative methods of intesive insulin therapy have not been evaluated in older adults with T2DM.
Objective: To compare the efficacy and safety of continuous subcutaneous insulin infusion (CSII) and multiple daily injections (MDI) in older adults with insulin-treated type 2 diabetes and to assess treatment satisfaction and quality of life.
Research Design and Methods: Adults (n = 107) 60 years of age and older (mean age 66 years) with insulin-treated type 2 diabetes (mean duration 16 years, mean body mass index 32 kg/m^2 and mean A1c 8.2%) were randomized to CSII (using insulin lispro) or MDI (using insulin lispro and insulin glargine) in a two-center, 12-month, prospective, randomized, controlled, clinical trial. Efficacy was assessed with A1c, safety by frequency of hypoglycemia, and treatment satisfaction and quality of life with the Diabetes Quality of Life Questionnaire and the SF-36.
Results: Forty-eight CSII subjects (91%) and 50 MDI subjects (93%) completed the study. Mean A1c fell by 1.7 ± 0.1% in the CSII group to 6.6% and by 1.6 ± 0.2% in the MDI group to 6.4%. The difference in A1c between treatment groups was not statistically significant (p=0.19). Seventy-five percent of subjects in the CSII group and 84% of those in the MDI group achieved A1c levels < 7.0% (p=0.30). Eighty-one percent of CSII subjects and 90% of MDI subjects experienced at least one episode of minor (self-treated) hypoglycemia (p=0.17) and three CSII and six MDI subjects experienced severe hypoglycemia (p=0.49). Rates of severe hypoglycemia were similarly low in the two groups (CSII, 0.08 and MDI, 0.23 events per person-year, p=0.61). Weight gain did not differ between groups (p=0.70). Treatment satisfaction improved significantly with both CSII and MDI (p<0.0001) and the difference between groups was not statistically significant (p=0.58).
Conclusions: In older subjects with insulin-treated type 2 diabetes, both CSII and MDI achieved mean A1c levels <7.0% with good safety and patient satisfaction.
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Optimising glycaemic control with BIAsp30 (once or twice daily) in combination with oral antidiabetic agents in type 2 diabetes

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Background and Aims: The premixed insulin analogue, biphasic insulin aspart 30 (BIAsp30, NovoMix® 30) consists of 30% soluble and 70% protamine-bound insulin aspart. This study was designed to evaluate the efficacy and safety of adding BIAsp30 versus optimising oral treatments in type 2 diabetes subjects inadequately controlled with oral monotherapy or combination therapy.
Materials and Methods: This 24-week, multi-national, multi-centre, open-labeled, randomised 2-arm parallel trial consisted of a 2-week screening period, followed by 24 weeks of treatment. Subjects randomised to BIAsp30 treatment received BIAsp30 once-daily (o.d.) at dinnertime for 12 weeks, with those not achieving an HbA_{1c} of lower than 8.5%, or having an HbA_{1c} of between 7 to 8.5% (both inclusive) and a fasting plasma glucose (FPG) level of >7 mmo/l, being switched to twice-daily (b.i.d.) BIAsp30 at midterm.
Results: A total of 173 subjects completed the trial. A significantly greater reduction in HbA_{1c} from baseline was seen at 11 weeks with BIAsp30 (o.d.) versus OAD-only treatment (1.16 ± 1.01 vs 0.58 ± 0.95% [p<0.001]; mean baseline HbA_{1c}: 8.61 and 8.46%, respectively), and this trend continued at 24 weeks, with significantly greater reductions in HbA_{1c} from baseline observed with BIAsp30 (o.d.) and BIAsp30 (b.i.d.) treatments versus the OAD-only treatment (1.24 ± 1.04 vs 1.34 ± 1.33 vs 0.67 ± 1.18%; p<0.01). Of the patients in the BIAsp30 (o.d.) group, 46% of patients and an additional 24% from the BIAsp30 (b.i.d.) group achieved the HbA_{1c} target of <7.0%, as compared to 29% of subjects on OAD-only treatment. Consistent with the trends in HbA_{1c}, significantly greater reductions in FPG from baseline was seen at 11 weeks with BIAsp30 (o.d.) versus OAD-only treatment (1.91 ± 2.22 vs 1.01 ± 2.20 mmol/l [p<0.05], as well as at 24 weeks, with BIAsp30 (b.i.d.) versus OAD-only treatment (2.32 ± 3.13 vs 1.10 ± 2.37 mmol/l [p<0.05]. A total of 178 episodes were reported by 69 patients (54%) treated with BIAsp30 and 46 episodes by 19 patients (30%) treated with OAD-only, of which all were minor or symptomatic, except for one in each treatment group which was major. Treatment emergent adverse events (TEAEs) were similar between BIAsp30 and OAD-only treatment. There were 5 serious TEAEs in the BIAsp30 group and none in the OAD-only group. None of these serious TEAEs were likely to be related to the trial product.
Conclusion: BIAsp30 provided greater glycaemic reductions than optimising oral treatment without increasing the risk of major hypoglycaemia. We conclude that the better option for type 2 diabetes subjects inadequately controlled with oral monotherapy or combination therapy is to initiate insulin treatment with BIAsp30 than to continue with oral antidiabetic treatment alone.
The study was sponsored by Novo Nordisk

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Treatment with biphasic insulin aspart 30/70 versus insulin glargine in insulin-naïve type 2 diabetes patients: long-term effects on diabetes-related complications

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Background and Aims: The aim of this study was to project the short-term clinical outcomes recently reported in the INITIATE clinical trial, to evaluate long-term effects on diabetes-related complications of treatment with biphasic insulin aspart 30/70 (BIAsp 30/70) versus insulin glargine in insulin-naïve type 2 diabetes patients failing oral antidiabetic agents.
Materials and Methods: The CORE Diabetes Model, a published, peer-reviewed and validated model of diabetes, was used to evaluate the cumulative incidence of diabetes-related complications over patient lifetimes (35 years). The model simulated the range of diabetic complications and disease progression within a series of sub-models (cardiovascular disease, neuropathy, renal and eye disease) based on published data. Baseline cohort characteristics (54.5% male, mean age 52.45 years, mean duration of diabetes 9 years, mean HbA1c 9.77%) and treatment effects (mean reduction HbA_1c 2.79 ± 0.11% with BIAsp 30/70 versus 2.36 ± 0.11% with glargine, p<0.01) were based on the INITIATE study. No discounting was applied. Cumulative incidence data for selected complications were presented with percentage reductions calculated relative to the cumulative incidence values in the glargine arm. Sensitivity analyses were performed.
Results: BIAsp 30/70 treatment was associated with substantial reductions in retinopathy and nephropathy complications compared to glargine. For example, the cumulative incidence of proliferative diabetic retinopathy was 3.9 ± 0.6% with BIAsp 30/70 treatment compared with 4.5 ± 0.7% with glargine. The cumulative incidence of end-stage renal disease, one of the costliest complications associated with diabetes, was only 6.8 ± 0.8% in the BIAsp 30/70 group compared with 8.1 ± 0.9% in the glargine group. Cumulative incidence rates for cardiovascular and other complications were comparable in both treatment arms.
Conclusion: This analysis is one of the first to address the long-term implications of treating type 2 diabetes patients failing oral antidiabetics with a premixed analogue insulin versus long-acting insulin. Based on the findings of the INITIATE study, our projections indicate that treatment with BIAsp 30/70 is associated with a substantially reduced cumulative incidence of diabetes-related complications compared to insulin glargine over patient lifetimes, particularly with respect to retinopathy and nephropathy complications.

Cummulative incidence over patient lifetimes (%)

	BIAsp 30/70 (mean±SD)	Glargine (mean±SD)	Percentage change with BIAsp 30/70
Proliferative diabetic retinopathy	3.9 ± 0.6	4.5 ± 0.7	–14%
End-stage renal disease	6.8 ± 0.8	8.1 ± 0.9	–16%
Lower extremity amputation	9.3 ± 1.0	9.5 ± 1.1	–2%
Neuropathy	59.7 ± 1.8	62.7 ± 1.8	–5%
Peripheral vascular disease	19.3 ± 1.3	20.9 ± 1.3	–8%
Congestive heart failure	39.5 ± 1.6	40.0 ± 1.6	–1%
Myocardial infarction	37.3 ± 1.5	38.7 ± 1.6	–3%
Stroke	13.9 ± 1.1	13.5 ± 1.1	+16%

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Similar glycemic control is achieved after discontinuation of thiazolidinediones and metformin in patients initiating insulin glargine as add-on therapy for type 2 diabetes: the GOAL A1C study

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Background and Aims: GOAL A1C (Glycemic Optimization with Algorithms and Labs At Po1nt of Care) was a 24-week randomized, parallel-group, open-label study designed to evaluate the addition of insulin glargine inpatients with type 2 diabetes (N=7,893) who were inadequately controlled on oral therapy alone (A1C>7%). The results of the primary comparisons are reported elsewhere. Here we report the effects of thiazolidinedione (TZD) and metformin discontinuation on efficacy and safety parameters in patients randomized to usual titration monitoring (no unsolicited contact between visits) and laboratory A1C evaluation.
Materials and Methods: Per protocol, TZDs were discontinued at randomization due to concerns about concomitant use with insulin at the time of the study. In addition, metformin was discontinued in patients with renal contraindications prior to study entry (ie, serum creatinine level ≥1.5 mg/dL in men or ≥1.4 mg/dL in women). Insulin glargine was added to existing oral therapy at 10 U/d and titrated using a simple algorithm based on self-monitored fasting blood glucose (FBG). Patients were evaluated in the clinic every 6 weeks.
Results: A total of 1491 patients were randomized to the usual insulin titration monitoring with laboratory A1C testing study arm. At study entry, the mean patient age was 57 years, BMI 34.5 kg/m^2, and average diabetes duration was 8.4 years. The majority of these patients were on combination therapy at baseline, most commonly a sulfonylurea plus metformin (39%), followed by a sulfonylurea plus metformin and a TZD (18%). The effect of TZD and/or metformin discontinuation on efficacy and hypoglycemia incidence is summarized in the table.

	Usual titration-lab A1C (n=1491)	Drug Discontinued TZD (n=177)	Metformin (n=57)	TZD + Metformin (n=24)
A1C*, %				
Baseline (BL)	8.8 ± 1.5	8.9 ± 1.5	9.0 ± 1.8	9.2 ± 1.6
Change from BL	–1.3 ± 1.5	–1.1 ± 1.5	–1.2 ± 1.5	–0.9 ± 1.8‡
FBG*, mg/dL				
BL	210 ± 69	202 ± 63	215 ± 78.0	231 ± 6.0
Change from BL	–77 ± 75	–62 ± 74	–83 ± 80	–93 ± 72
Insulin Glargine dose at 24 weeks*, U	50 ± 35	59 ± 39	48 ± 30	61 ± 27
Weight change*, kg	1.9 ± 7.4	1.7 ± 7.5	2.7 ± 5.6	3.2 ± 3.3
Hypoglycemia§				
BG<70 mg/dL	3.7	3.9	4.4	1.3
Severe	0.09	0.12	0.0	0.0

* Values are mean±SD; *P*<.0001, baseline vs end of study; ‡ *P*=.018, baseline vs end of study; § events per exposure year.

Conclusions: Overall rates of hypoglycemia were low. Despite discontinuation of TZDs and metformin in some patients, similar significant improvements in glycemic control were achieved over the 24-week study period with the addition of insulin glargine using a simple titration algorithm in a mainly primary care setting.
This study was supported by the sanofi-aventis Group

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Glucose control during early insulinization with glargine as assessed by the continuous glucose monitoring system (CGMS)

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Background and Aims: Insulin is generally withheld from people with type 2 diabetes until they are unresponsive to other therapies. However, potential advantages of insulin suggest that it may have a role in early management.
Materials and Methods: Adults with type 2 diabetes on stable therapy with 0, 1 or 2 oral agents, whose A1c was 7.5%–11%, and who were not taking a thiazolidinedione were allocated to either the addition of evening glargine insulin or optimization of oral agents (OAD) for the next 24 weeks. Both groups targeted A1c levels <7%. CGMS monitoring and a 7-point home blood glucose monitoring (HBGM) log were performed in 99 patients at baseline, 56, 84 and 168 days. For the CGMS monitoring a hypoglycemia episode is defined by observing 3 consecutive values of glucose monitoring <4.0 mmol/L until the next 3 consecutive values of glucose monitoring ≥4 mmol/L. To assess the accuracy of the CGMS, 4067 pairs of simultaneous glucose level determinations were compared between CGMS and HBGM.
Results: 206 participants were allocated to glargine and 199 to oral agents. The A1c decreased more significantly in the glargine than the OAD group (–1.55 vs –1.25% (p=0.0047), as did fasting plasma glucose (–3.89 vs –2.31 mM, p=0.0001). The frequency of hypoglycemia at anytime of the day was 44/50 (88%) and 45/49 (92%) in the insulin and OAD treatment groups, respectively (p-value = 0.9957). Median total number of episodes per day was 0.81 (nighttime = 0.46, daytime = 0.26) and 0.72 (nighttime = 0.46, daytime = 0.24) for the glargine and OAD, respectively. The correlation between the HBGM and CGMS datapoints was 0.97 (p<0.001), (CGMS = 0.97 x HBGM + 0.25). Analysis using the Clarke error grid revealed 75.0% A, 23.2% B, 0.1% C, 1.6% D and 0.1% E.
Conclusion: Early addition of glargine insulin can safely reduce A1c levels more than continuing with diet or oral agents alone. The CGMS provides accurate monitoring of glucose levels through the entire glucose range.
Support: Aventis Canada

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Glycemic control achieved with insulin glargine as "add-on" therapy in type 2 diabetes mellitus is unaffected by age, race, diabetes duration or body mass index: The GOAL A1C Study

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Background and Aims: GOAL A1C (Glycemic Optimization with Algorithms and Labs At Po1nt of Care) assessed the impact of active (weekly patient contact) vs usual (no unsolicited contact between scheduled visits) monitoring of insulin titration and point-of-care (POC) vs laboratory A1C assessment (Lab) testing on glycemic control.
Materials and Methods: This 24-week, multicenter, open-label study conducted mainly by primary care physicians evaluated the addition of glargine in 7893 patients with type 2 diabetes who were not optimally controlled on oral agents (baseline A1C >7%). Patients were randomized to 1) usual insulin titration monitoring + lab A1C testing; 2) usual titration monitoring + POC A1C; 3) active titration monitoring + lab A1C; or 4) active titration monitoring + POC A1C. We examined the influence of age, race, diabetes duration, and BMI on glycemic control.
Results: The table summarizes the impact of various demographic and baseline disease characteristics on change in A1C from baseline to endpoint in patients for whom complete data was available (N=5721).

Mean Change (Δ) in A1C (%) from Baseline (BL)*

	N	Usual-Lab (n=1487)		Usual-POC (n=1362)		Active-Lab (n=1498)		Active-POC (n=1364)	
		BL	Δ	BL	Δ	BL	Δ	BL	Δ
Overall	5721	8.8	−1.3	8.9	−1.3	8.9	−1.5	8.8	−1.5
Age, years									
<65	4241	9.0	−1.4	9.0	−1.4	9.1	−1.7	9.0	−1.7
≥65	1470	8.5	−1.0	8.6	−1.0	8.6	−1.3	8.4	−1.1
Race									
White	4034	8.7	−1.2	8.7	−1.2	8.7	−1.4	8.6	−1.5
Black	914	9.4	−1.5	9.6	−1.9	9.5	−1.9	9.3	−1.7
Hispanic	587	9.2	−1.5	9.1	−1.4	9.3	−1.7	9.2	−1.8
Other	172	8.6	−1.1	8.7	−1.2	9.8	−2.4	8.9	−1.2
Duration of Diabetes, years									
<5	1653	8.8	−1.4	9.0	−1.6	8.8	−1.6	8.9	−1.8
≥5	4037	8.9	−1.3	8.9	−1.2	9.0	−1.5	8.8	−1.4
BMI, kg/m^2									
<28	1096	9.0	−1.5	9.0	−1.4	9.2	−1.8	9.0	−1.7
28≤BMI<40	3322	8.8	−1.3	8.9	−1.3	8.9	−1.5	8.8	−1.5
≥40	1176	8.8	−1.1	9.0	−1.3	8.8	−1.3	8.8	−1.5

*P<.01 for all age, race, diabetes duration, and BMI subgroups in all study arms

Conclusions: With the simple addition of insulin glargine, significant reductions in A1C from baseline to endpoint can be achieved in a predominantly primary care setting regardless of baseline age, race, BMI, duration of diabetes, or baseline A1C. Although not statistically significant, greater reductions in A1C were associated with age <65 years, BMI <28 kg/m^2, and duration of diabetes <5 years.

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Clinical aspects of basal insulin pump therapy for 8 hours versus 24 hours in patients with type 2 diabetes

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Background and Aims: Basal continuous subcutaneous insulin infusion (CSII) therapy may improve glycemic control in type 2 diabetic patients with oral treatment failure. The aim of the present study was to compare the effect of basal CSII therapy for 8 hours at night and 24 hours, employing the short-acting insulin aspart analog together with oral anti-diabetic (OAD) medication.

Materials and Methods: Ten type 2 diabetic patients with poor plasma glucose control in response to OAD treatment (fasting plasma glucose (FPG) 9–16 mmol/L, HbA_{1c} >7.5%) were included in this open-label, randomized cross-over study. Following an initial 24 hours control day, two 3-day periods with basal CSII therapy (insulin aspart 1.5 IU/h) for 8 hours during the night versus 24 hours, were compared, separated by a 2 week wash out period. The patients received usual OAD treatment throughout the study. Plasma glucose and serum insulin were recorded before and after each of three meals, at bedtime, and at night. The primary endpoints were FPG and post-prandial plasma glucose (0–2 h).

Results: Compared to the control day, the 8 hours infusion period significantly improved FPG (3.28 ± 0.59 mmol/L; P <0.0001) and post-prandial plasma glucose after breakfast (3.32 ± 0.62 mmol/L, P <0.0001), whereas 24 hours infusion improved as well FPG (3.83 ± 0.59 mmol/L, P <0.0001) as all 3 post-prandial plasma glucose values (4.81 ± 0.59 mmol/L, 2.46 ± 0.60 mmol/L, 2.69 ± 0.59 mmol/L; all P <0.0001). Moreover, compared with the 8 hours infusion, the 24 hours infusion also significantly improved plasma glucose after breakfast (1.49 ± 0.46 mmol/L; P = 0.0014), after lunch (1.60 ± 0.47 mmol/L; P = 0.0007), and after dinner (1.84 ± 0.46 mmol/L; P = 0.0001). Two patients experienced a single hypoglycemic episode (plasma glucose <3.5 mmol/L), and one had three episodes.

Conclusion: Additional treatment with basal CSII therapy for 8 hours nightly and 24 hours daily, employing insulin aspart infusion at a rate of 1.5 IU/h, provided efficacy and safety in type 2 diabetics receiving usual OAD treatment. A 24 hours infusion was increasingly superior to infusion for 8 hours during the night regarding post-prandial glucose control, but the insulin dose administered was also tripled. Basal CSII therapy could be considered on an individual basis when initiating insulin combination therapy in patients with type 2 diabetes.

Support: Novo Nordisk A/S

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Insulin lispro efficacy: linkage with other antidiabetics in patients with type 2 diabetes – the "HELP 2" observational study

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Background and Aims: The initiation of insulin therapy in type 2 diabetes is mostly triggered by surrogate parameters like HbA1c. Little is known about the influence of antidiabetic pre- and concomitant treatment. Therefore, in an observational study, we evaluated effectiveness and tolerability of insulin lispro (LP) injections in patients with type 2 diabetes on varied pretreatments.

Materials and Methods: In a total of 1018 sites, 4062 type 2 diabetic patients with at least 2 years of antidiabetic pretreatment were observed after starting prandial administration of LP. The collected parameters were HbA1c, lipid profile, BMI, hypoglycemic and adverse events. A descriptive statistical analysis was performed.

Results: The patients (male/female = 51.2/48.8%) had a mean age of 59.5(± 11.6) years and a mean BMI of 29.3(± 4.9) kg/m^2. In terms of concomitant therapy, LP was used in 11.3% as monotherapy, in 16.5% combined with oral antidiabetics (OADs), in 44.8% combined with other insulins and in 27.4% combined with other insulins and OADs. During the mean observation interval of 9.3(± 4.4) weeks HbA1c decreased by 1.45(± 1.16)%, triglycerides by 40.7(± 93.2) mg/dl and cholesterol by 19.3(± 39.1) mg/dl in the study population. Body weight decreased slightly by –0.3(± 3.5) kg (95%CI [–0,19; –0,31]) in the overall cohort, whereby this reduction was the more pronounced in the LP monotherapy group (–0.6 ± 2.34 kg; 95% CI [–0.38; –0.82]). In terms of pretreatment, OAD pretreatment was most commonly given (45.9%). Analyses per pre-treatment are summarized below (Table).

Parameter	Pretreatment Dietetic only (N=187)	OAD (N=1864)	Insulin (N=1143)	Insulin + OAD (N=868)
HbA1c [%]	Baseline	8.96 ± 1.91	9.00 ± 1.37	8.76 ± 1.36
	Change	-1.78 ± 1.58	-1.60 ± 1.17	-1.34 ± 1.09
	95%CI Change	[-1.52; -2.04]	[-1.54; -1.66]	[-1.26; -1.42]
Post breakfast blood glucose excursion [mg/dl]	Baseline	42.61 ± 52.82	46.10 ± 42.31	44.49 ± 45.85
	Change	-23.82 ± 51.38	-25.52 ± 43.17	-22.68 ± 46.76
	95%CI Change	[-15.62; -32.02]	[-23.39; -27.65]	[-19.31; -26.04]
BMI [kg/m^2]	Baseline	28.11 ± 5.28	29.39 ± 4.68	30.43 ± 5.04
	Change	-0.17 ± 1.12	-0.08 ± 1.04	-0.20 ± 1.01
	95%CI Change	[-0.01; -0.33]	[-0.03; -0.13]	[-0.13; -0.27]

The number of hypoglycemic episodes increased slightly during the observation, whereas the number of severe hypoglycemic episodes decreased.

Conclusion: Prandial LP treatment was well tolerated in a naturalistic setting. Results indicate an improvement of blood glucose level and HbA_1c. The results of the subgroup-analyses have to be interpreted carefully, because they can be confounded in the observational setting, but they can help to generate hypotheses for further controlled clinical trials.

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Insulin treatment in type 2 diabetes IV

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Biphasic insulin aspart 30, a premix analogue, is an effective and well-tolerated starter insulin in type 2 diabetes

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Background and Aims: Due to the progressive nature of the disease, patients with type 2 diabetes need regular monitoring and their treatment often requires intensification once an existing treatment regimen fails to meet glycaemic targets. The efficacy, tolerability and acceptance of two regimens of biphasic insulin aspart 30 (BIAsp 30; 30% rapid-acting insulin aspart and 70% intermediate-acting insulin aspart) were evaluated in an open-labelled, comparative, multicentre study in Russia.
Materials and Methods: A total of 308 patients with poorly-controlled type 2 diabetes receiving one or more oral antidiabetic drugs (OADs) (mean HbA_{1c} 10.3%), were randomised to three treatment arms: group 1 - BIAsp 30 three times daily (TID); group 2 - BIAsp 30 twice daily (BID) + metformin, and group 3 - OAD treatment continued and optimised.
Results: After 16 weeks, glycaemic control improved in all groups; however, both BIAsp 30 groups (1 and 2) consistently achieved significantly better glycaemic control compared with OAD monotherapy (3). There was a greater reduction in HbA_{1c} (-2.88%; -3.01%; -2.05%, respectively; 1 vs 3, P<0.001; 2 vs 3, P<0.001) and a higher percentage of patients achieved HbA_{1c} target of <7% (42.0%; 45.0%; 26.2%, respectively; 1 vs 3, P=0.012; 2 vs 3, P=0.02). Furthermore, there was a greater reduction in mean plasma glucose value, based on 7-point plasma glucose profile in groups 1 and 2 (-5.91; -5.52; -3.49 mmol/l, respectively; 1 vs 3, P<0.001; 2 vs 3 P<0.001). A greater reduction in mean prandial plasma glucose increment was seen with BIAsp 30 (BID) + metformin than the other two treatment groups (–1.26; –2.15; -0.44 mmol/l for groups 1, 2 and 3, respectively; 1 vs 3, P=0.047; 2 vs 3, P<0.001; 1 vs 2, P=0.042). Mean insulin doses in groups 1 and 2 were 55.5 ± 16.2U and 44.8 ± 12.6U, respectively. Improvement in glycaemic control was associated with slight weight changes in all treatment groups (1.71; 1.54; -0.79 kg) and some increase in minor hypoglycaemia (groups 1 vs 2, P=NS). No major hypoglycaemic episodes were reported. Treatment satisfaction, based on the WHO Diabetes Treatment Satisfaction Questionnaire, increased in all treatment groups with tendency to higher satisfaction in both insulin treatment groups compared with OAD treatment.
Conclusion: BIAsp 30 TID and BIAsp 30 BID + metformin significantly improve glycaemic control in patients with type 2 diabetes in poor control in just 16 weeks. Furthermore, it is an acceptable treatment regimen by patients. A slight increase in body weight and incidence of minor hypoglycaemia with insulin treatment groups does not influence patients' treatment satisfaction
Support: Novo Nordisk

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Biphasic insulin aspart given thrice-daily is as efficacious as a conventional basal bolus insulin regimen with four daily injections in subjects with type 2 diabetes

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Background and aims: Biphasic insulin aspart (BIAsp) 30, 50, and 70 are premixed forms of insulin aspart (IAsp), differing in the ratio between soluble, rapid-acting IAsp and protamine-bound, intermediate-acting IAsp. The number denotes the percentage of soluble IAsp. These products have been developed to overcome the shortcomings of conventional basal-bolus insulin regimens by more closely matching meal-time physiological insulin secretion while also providing basal insulin coverage. BIAsp 30 has been shown to provide superior postprandial glycaemic control to biphasic human insulin 30 in a twice-daily treatment regimen. However, some patients with type 2 diabetes will eventually need to intensify their insulin regimen to achieve a good control. BIAsp 50 and BIAsp 70 offer a new possibility for intensification of once- or twice-daily insulin therapy to a thrice-daily insulin regimen without increasing the risk of late postprandial hypoglycaemia. This provides patients a more convenient alternative to a conventional basal bolus therapy which requires at least 4 daily injections. The aim of this multinational, randomised, open-label parallel-group 16-week trial was to show that a thrice-daily meal-time BIAsp treatment regimen is as efficacious as a conventional 4 times daily basal bolus regimen with human isophane insulin (NPH) and IAsp.
Materials and methods: Subjects with type 2 diabetes (BMI ≤35 kg/m^2, 8.0% ≤HbA_{1c}≤10.5%) on once or twice daily insulin regimens were randomised 1:1 to either BIAsp or IAsp+NPH for 16 weeks. Treatment in the BIAsp group (n=196) was tailored according to individual needs using BMI as surrogate index of insulin resistance. Subjects administered BIAsp 70 (BMI ≤30 kg/m^2) or BIAsp 50 (BMI >30 kg/m^2) with breakfast and lunch and BIAsp 30 with dinner. The IAsp+NPH group (n=198) injected IAsp at meals and NPH as basal insulin. Titration in both groups was aiming at fasting plasma glucose (FPG) <7.2 mmol/L and peak post prandial plasma glucose (PPG) <10.0 mmol/L. The primary endpoint, HbA_{1c} levels after 16 weeks, was compared between treatments using a predefined non-inferiority criterion of 0.4%. Safety was evaluated in terms of the incidence of hypoglycaemic episodes and adverse events.
Results: Mean HbA_{1c} (±SD) decreased from 9.1 ± 0.7% to 7.8 ± 1.0% with both treatments. Glycaemic control, as measured by HbA_{1c}, provided by BIAsp was non-inferior to that obtained by IAsp+NPH (diff -0.05; 95% CI [-0.24; 0.14]). Similar improvements in glycaemic control in both groups were confirmed by self-measured 8-point plasma glucose (PG) profiles, average PG concentrations, and average prandial PG increments. The incidence of adverse events and hypoglycemic episodes was similar in the two treatment groups.
Conclusions: The results of this trial show that a thrice-daily BIAsp regimen (BIAsp 70/70/30 or BIAsp 50/50/30) can be used as an intensive therapy in people with inadequately controlled type 2 diabetes, with fewer daily injections than a conventional basal-bolus therapy, and without compromising efficacy or safety.

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Efficacy and safety of glargine insulin in primary care

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Background and Aims: Most patients with type 2 diabetes (T2DM) are cared for by a family physician (FP). Many of these patients ultimately require exogenous insulin to achieve and maintain optimal glycemic control. Most FPs are unfamiliar with or hesitant to use insulin. Development and evaluation of strategies to facilitate safe and efficacious use of insulin by FPs are needed.
Materials and Methods: A national, multicentre randomized trial was designed to assess early insulinization using bedtime insulin glargine vs a standard oral agent strategy for patients with T2DM. 19 endocrinologist/expert and 34 FP sites with little expertise in insulin initiation were recruited. FPs were provided with CME and resources to make them confident and comfortable with insulin initiation and therapy. Consenting patients aged 18–80 with T2DM for at least 6 mths on stable therapy with 0, 1 or 2 oral agents (excluding TZDs) and an HbA_1c of 7.5–11% were randomized to either the addition of nightly glargine insulin (with no change in oral therapy) or optimization of oral therapy (with no insulin). 145 patients were followed by experts and 260 by FPs. All patients on glargine followed a standard protocol to increase doses by 1 unit/day until FPG levels were <5.5 mmol/L. The HbA_1c target for both groups was 7% or less. The primary measure was % of subjects in each treatment arm who achieved 2 or more consecutive A1Cs of 6.5% or less.
Results: 206 of 614 screened patients were randomized to the glargine arm and 199 to the oral agent arm. Mean baseline HbA_1c was 8.6% and mean FPG was 10.7 mmol/L. There was no statistical difference in baseline BMI or HbA_1c for FP and expert patients. The direction of the treatment effect in favour of glargine was the same for FPs and experts, with no statistically significant interaction between FPs/experts and treatment for the primary outcome (Table 1). Mean HbA_1c and FPG reduction, and rates of hypoglycemia were comparable between patients of FP and experts. Both FPs and experts achieved significantly greater reductions in FPG with glargine than with oral agents (FPs: –4.13 vs –2.38 mmol/L, p=0.0001; Experts: –3.47

vs –2.19 mmol/L, p=0.0013). However, FPs also achieved significantly greater reductions in HbA_1c with glargine than with oral agents (FPs: –1.64 vs -1.26%, p=0.0058; Experts: -1.41 vs -1.23%, p=0.3038). Final mean insulin dose was higher in FP than expert patients (41.74 vs 31.66 units [95% CI, 1.98–18.17; p=0.015]).
Conclusion: FPs achieved glycemic targets more effectively with glargine than with standard lifestyle or oral agent therapy. When provided with training in insulin initiation and therapy and simple algorithm for patient use, FPs prescribed basal insulin as safely and effectively as diabetes experts. The fact that the mean insulin dose was higher in FP patients than experts patients indicates that FPs were comfortable with aggressive insulin use to achieve and sustain glycemic targets.

Table 1. Patients (n and %) achieving and sustaining (2 or more consecutive values) glycemic targets

	FP G n (%)	OA n (%)	HR (95% CI)	P	GP G n (%)	OA n (%)	HR (95% CI)	P	P of interaction
Sustained A1C =<6.5%	27 (20.6)	17 (13.2)	1.69 (0.92, 3.09)	0.0916	11 (14.7)	6 (8.6)	1.74 (0.64, 4.69)	0.2779	0.9558
Sustained A1C <=7%	63 (48.1)	39 (30.2)	1.86 (1.25, 2.77)	0.0024	33 (44)	23 (32.9)	1.36 (0.80, 2.32)	0.2566	0.3511
First A1C <=6.5%	47 (35.9)	42 (32.6)	1.21 (0.79, 1.84)	0.3797	20 (26.7)	11 (15.7)	1.61 (0.77, 3.37)	0.2045	0.5053
First A1C <=7%	91 (69.5)	67 (51.9)	1.65 (1.20, 2.27)	0.0019	45 (60)	36 (51.4)	1.19 (0.77, 1.84)	0.4404	0.2202

ITT=intent-to-treat A1C=glycated hemoglobin G=glargine insulin OA=oral agents HR=hazard rate

Sponsored by sanofi-aventis

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Insulin glargine improves health related quality of life in patients with type 2 diabetes inadequately controlled on sulfonylurea plus metformin
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Background and Aims: Recently, we reported the primary outcomes of a 24-week multicenter, randomized, parallel group trial evaluating add-on insulin glargine vs rosiglitazone in 217 patients with type 2 diabetes who were on a sulfonylurea plus metformin (SU+MET) (ADA 2004). Comparable glycemic control and hypoglycemia profiles were observed with both regimens. We tested the hypothesis that add-on insulin glargine improves health-related quality of life (HRQOL) with equivalent glycemic control.
Materials and Methods: The Diabetes Symptom Checklist-Revised (DSC-R; 8 symptom and 8 distress sub-scales; 5-item emotional well-being, 1-item general health perception scale adapted from SF-36) was administered at baseline, and at weeks 2, 6, 12, 18, and 24.
Results: At baseline, before being added to the study, insulin glargine patients scored poorer than rosiglitazone patients in all DSC-R symptom and distress domains. HRQOL improved from baseline in both groups. However, greater HRQOL improvements from baseline were achieved for patients randomized to insulin glargine vs rosiglitazone (table). There were no significant differences between the 2 treatments for any other domains.

	Glargine – rosiglitazone	*P*-value
Total symptoms	-7.59	.005
Total distress	-1.92	.03
General health	-5.38	.047
Hypoglycemia symptoms	-12.86	.007
Visual disturbance	-7.59	.005
Visual dysfunction distress	-3.14	.013
Fatigue	-4.98	.033

When evaluating HRQOL domains against clinical outcomes, poorer HRQOL was consistently associated with early study termination (n=8, insulin glargine; n=21, rosiglitazone). Higher A1C, even when controlled for hypoglycemia and early termination, was associated with lower general health (*P*=.008) and emotional well-being (*P*=.013), increased hyperglycemia distress symptoms, fatigue (*P*=.0003 and .008) and general distress (*P*=.0002).
Conclusions: In patients who failed to achieve glycemic goals with SU+MET therapy, add-on insulin glargine was associated with significantly greater improvement in symptoms and distress and HRQOL over 24 weeks vs add-on rosiglitazone despite equivalent improvement in glycemic control. While improved glycemic control enhances HRQOL, aspects of insulin therapy may enhance HRQOL independent of glycemic control. DSC-R is a sensitive HRQOL instrument for evaluating treatment response of patients with type 2 diabetes and may help select patients who are likely to benefit from insulin-oral combination therapy.
This study was supported by Aventis Pharmaceuticals, a member of the sanofi-aventis Group

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Risk of exercise induced hypoglycemia in patients with diabetes mellitus type 2 (DM2) on intensified insulin therapy (ICT). Comparison of NPH-insulin versus glargine as basal insulin supplement
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Background and Aims: Insulin glargine is a long-acting recombinant human insulin analogue with a duration of action of up to 24 hours. Therefore adjustment of the insulin dose due to physical activity may be difficult. Thus we compared the therapy-related risk of hypoglycemia in patients on insulin glargine or NPH-insulin, as basal insulin, during a treadmill-exercise test.
Materials and Methods: 123 patients (female 37) with DM2 were evaluated. All patients were on ICT and had either Neutral Protamine Hagedorn insulin (NPH) or Glargine (GLA) as basal insulin supplement. All data are median (25./75. percentile). HbA1c: 7.5% (6.9/7.9) and 7.3% (6.8/7.9), p = 0.16; age: 63 (57/69) and 61 (55/64) years, p = 0.001. Bodymass index: 31.5 (28.5/34.8) and 30.9 (27.2/34.5), p = 0.02; NPH and GLA, respectively for each. Blood glucose (BG) and capillary lactate concentration (LAC) were measured at 10 min intervals during a 30 min exercise test at 60% of maximal oxygen consumption (VO2 max). At least 2 days before the study, patients performed a pre-test to determine the physical workload below the anaerobic threshold at a target LAC of 2.0 mmol/l. During both tests velocity and inclination were adjusted according to LAC. The results of the pre-test were used at the beginning of the exercise test. BG was also recorded as three 8-point/d BG-profiles, starting one day before the study.
Results: 120 patients completed the exercise test, 62 patients with NPH and 58 with GLA. The BG (values in mg/dl) at the beginning [BG (0 min)] of the exercise test, minimal BG [BG (Min)] and the BG decline [BG (dec)] were comparable in both groups, [BG (0 min) 138 (113/146) vs 129 (113/141), p = 0.35, BG(Min): 87 (72/105) vs 78 (71/103), p = 0.29; BG (dec) 40 (27/56) vs 45 (25/53), p = 0.68, NPH and GLA, respectively]. LAC (mmol/L) at beginning and maximal LAC were not significantly different in both groups [LAC (0 min): 1.40 (1.19/1.75) vs 1.49 (1.12/1.83), p = 0.67 and LAC (max) 1.92 (1.60/2.25) vs 2.0 (1.64/2.40), p = 0.09, NPH and GLA, respectively]. Maximal LAC were reached at 4.1 km/h (3.0/5.3) and 1.5% (1.5/2.5) inclination with NPH and 4.6 (4.0/5.5) km/h and 2.5 (1.5/4.0) % inclination with GLA, p = not significant. During 120 exercise tests the incidence of hypglycemia was 2.5%. Three episodes of mild hypoglycemia (BG < 60 mg/dl, with symptoms) occurred in the GLA-group, none in the NPH-group. Additional carbohydrate intake due to mild symptoms of hypoglycaemia with BG >60 mg/dl and <90 mg/dl, was recorded 9 times with NPH and 17 times with GLA, again, the difference was not significant. After the exercise tests no more episodes of hypoglycaemia were detected during follow up or in the 8-point/d BG-profiles.
Conclusion: Physical inactivity plays a major role in the development of the metabolic syndrome and DM2. Therefore as expected, the physical workload to achieve a lactate levels >2 mmol/l was low in the study population, indicating the lack of physical training. In the Glargine group additional carbohydrate intake was higher, initial BG and minimal BG were slightly lower than in the NPH group, but none of the differences were statistically significant. The risk of hypoglycaemia during physical activity is low in patients with Diabetes mellitus type 2 treated with ICT independent of the basal insulin used.
The study was supported by Aventis

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Comparison of premeal insulin lispro plus bedtime NPH with twice-daily NPH: effects on glycemic control and cardiovascular risk factors in type 2 diabetes

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Background and aims: To assess the effects of postprandial (pp) blood glucose (BG) changes on risk factors and possible surrogate markers linked to cardiovascular disease.
Materials and methods: In an open-label, crossover study, 30 patients (18 M, 12 F; mean age 61 yrs) with type 2 diabetes (mean duration 16 yrs) were randomized after an 8-week lead-in period with twice-daily NPH (post lead-in HbA1c: 8.37%± 0.94%). One group received a prandial regimen consisting of premeal insulin lispro (LP) plus bedtime NPH for 12 weeks followed by a basal regimen of twice-daily NPH for 12 weeks. The other group received the reverse treatment sequence. Endpoint values of glycemic control and key cardiovascular risk factors are expressed as mean±SEM. Due to the exploratory nature of this study, p-values <0.1 indicate a potential treatment difference.
Results:

	LP (N=29)	NPH (N=28)	p-value
HbA1c (%)	7.60 ± 0.15	8.18 ± 0.22	<0.001
Total daily insulin dose (U/kg)	0.45 ± 0.06	0.53 ± 0.07	0.052
D-glucitol (mg/dL)	4.88 ± 0.62	3.41 ± 0.35	0.011
Self BG monitoring *			
Mean premeal BG (mM)	8.78 ± 0.33	8.79 ± 0.37	NS
Mean 2-hr pp BG (mM)	9.18 ± 0.37	11.36 ± 0.38	<0.001
Hypoglycemia			
Incidence n(%)	6(22%)	8(30%)	NS
Nocturnal incidence n(%)	3(11%)	5(19%)	NS
Lipid parameters			
Cholesterol (mM)	5.49 ± 0.15	5.73 ± 0.21	0.049
LDL (mM)	3.47 ± 0.15	3.69 ± 0.19	0.053
Oxidized LDL (U/L)	75.0 ± 3.9	87.0 ± 7.5	0.043
Triglycerides (mM)	1.91 ± 0.26	1.68 ± 0.15	NS
HDL (mM)	1.23 ± 0.05	1.27 ± 0.06	NS

* Mean = the average of BG readings before or following the 3 main meals.
LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Conclusions: Compared with a twice-daily regimen of NPH, premeal insulin lispro with bedtime NPH significantly improved overall glycemic control and pp BG without increasing total daily insulin dose or incidence of hypoglycemia. Moreover, the cardiovascular risk profile was improved following treatment with the insulin lispro regimen, due to lower total cholesterol, LDL cholesterol, and oxidized LDL.

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Insulin glargine initiation in sub-optimally controlled patients with type 1 or 2 diabetes: sub-analysis of the AT.LANTUS trial investigating glycaemic control in the older patient population

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Background and Aims: This sub-analysis of a multi-centre, multi-national, 24-week, randomised, parallel study compared efficacy and safety of insulin glargine initiation in older type 1 (≥60 years) and Type 2 (≥70 years) diabetes patients versus the rest of the study population.
Materials and Methods: Titration was based on two treatment algorithms with a target fasting blood glucose (FBG) of 4.4–6.7 mmol/L (80–120 mg/dL) for type 1 patients and ≤5.5 mmol/L (≤100 mg/dL) for type 2 patients. Treatment groups were compared for incidence of hypoglycaemia (severe FBG <2.8 mmol/L [<50 mg/dL]) and glycaemic control (HbA_{1c} and FBG).
Results: In the type 1 population, there were no differences in baseline characteristics between the older patients and the younger patients (<60 years) except duration since diagnosis, which was significantly longer in the older population (27.0 vs 14.1 years; p <0.001). There was no difference between the older versus the <60 year population in terms of incidence of severe, symptomatic and nocturnal hypoglycaemia, baseline to endpoint change in HbA_{1c} or FBG (Table). There was a slight increase in body weight in both groups (Table). In the type 2 population, baseline characteristics did not differ between the older patients and the rest of the type 2 study population except duration since diagnosis (significantly longer in the older versus <70 year group: 15.5 vs 11.9 years; p <0.001) and FBG (significantly lower in the older group; p <0.001). There was no difference between the older versus the <70 year group in terms of incidence of severe, symptomatic and nocturnal hypoglycaemia (Table), again with similar outcomes per algorithm. HbA_{1c} and FBG decreased in both groups (Table). The fall in HbA_{1c} was significantly greater in the <70 year group (p <0.001); endpoint FBG was significantly lower in the older group (p <0.02). Body weight increased slightly in both groups (Table) and this increase was significantly greater in the <70 year group (p <0.001).
Conclusion: This sub-analysis shows that, in patients with type 1 or type 2 diabetes, improvements in glycaemic control can be obtained in poorly controlled older patient populations without an increased risk of hypoglycaemia. Thus, initiation of insulin glargine in older patients with type 1 or type 2 diabetes is as safe and effective as in younger patients.

		Type 1 (n=2140)		Type 2 (n=4588)	
		<60 years (n=2032)	≥60 years (n=108)	<70 years (n=4102)	≥70 years (n=486)
Hypoglycaemia (%)	Severe	7.2	8.3	1.0	1.0
	Symptomatic	80.2	75.0	28.3	25.5
	Nocturnal	22.0	27.8	3.8	2.7
HbA_{1c} (%)	Baseline	8.5	8.4	9.0	8.7
	Change	-0.7	-0.5	-1.2*	-0.8
FBG, mmol/L (mg/dL)	Baseline	9.9 (178.6)	9.6 (172.9)	9.5* (171.6)	8.5 (153.1)
	Change	-3.3 (-58.8)	-2.8 (-49.6)	-3.4 (-60.9)	-2.6 (-45.9)
Body weight (kg)	Baseline	70.1	72.8	80.3	75.7
	Change	0.7	0.7	1.2*	0.6

*p <0.001 between groups

This study was sponsored by sanofi-aventis.

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Pregnancy I

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Clinical outcome of pregnancies in women with type-1-diabetes. Did we achieve the St.-Vincent declaration?

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Background and Aims: Type-1-diabetes in pregnancy occurs in 2 of thousand pregnancies. It can result in significant short- and long term morbidity to mother and offspring. The 1989 St. Vincent's-Declaration stated that the risk of diabetic pregnancies should be lowered to the risk of non-diabetic women. The aim of our study was to compare pregnancy outcome between diabetic and non-diabetic pregnancies in our clinic.
Materials and Methods: Eightyseven pregnant diabetic were matched to 261 non-diabetic controls. The main outcome were the rate of stillbirth, congenital malformation, birth weight and transfer to the neonatal unit care as well as maternal morbidities like pregnancy induced hypertension and rate of operative delivery.
Results: Among 348 singleton-pregnancies we had 5 stillbirths, 4 of them in diabetic pregnancies. There were no significant differences in infant weights (3,5 vs. 3,1 kg) or macrosomia (13% vs. 12%). Congenital malformations in diabetic pregnancies occurred in 9.2% vs. 7.2% in the control group. The diabetes group had a significant higher frequency of maternal hypertension (6.8% vs. 0.7%), induction of labour (52% vs.17%), C-section (40% vs. 20%), preterm delivery (31% vs. 13%), neonatal hypoglycemia (65% vs. 5%) and admission to a neonatal unit (64% vs. 16%; $p<0.05$). The mean of the maternal HbA1c values decreased during pregnancy from 8.8% before pregnancy to 6.4% in the last trimenon. Short-acting insulin showed no increased malformation-rate or complications. There were no significant differences in birth weight (3,5 vs. 3,1 kg) or rate of macrosomia (13% vs. 12%).
Conclusion: Pregnancies complicated by Diabetes are still associated with a higher frequency of adverse maternal and fetal outcome. The outcomes seemed to be unaffected by treatment modality (insulin- pump or ICT). Insulin-Lispro seemed to help reducing hypoglycemia. However, the goals of St. Vincent- Declaration are still not achieved. The high pre-gestational HbA_1c showed that we have to bring the pre-gestational period in our focus.

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Outcome of pregnancy in T1DM: Results of Dublin Diabetes Pregnancy Audit (1998–2004)

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Background and Aims: The aim of this study was to compare the pregnancy outcomes in type 1 diabetic pregnancies (T1DMP) with the background population.
Materials and Methods: Data on 373 women with T1DM from the combined diabetes pregnancy clinics in 3 university maternity hospitals in Dublin over the last 6 years (1998–2004), were compared with total of 112,725 pregnancies delivered in the same hospitals for the same period.
Results: T1DM was present in 373 (0.3%) of total population T1DMP had a mean age of (30.4 ± 5.3 years), duration of diabetes was (12.9 ± 8.4 years) and booking gestation to the combined clinic was (8.4 ± 4.5 weeks). HbA_1c values decreased with advancing pregnancy: baseline (7.7 ± 1.5%); first trimester (6.6 ± 1%); second trimester (6.1 ± 0.8) at delivery (6.4 ± 0.3%). Gestation at delivery was (38 ± 2 weeks) with mean birth weight of (3.6 ± 0.7 Kg).The miscarriage rate was 18% in T1DMP compared with 8% in the control population. There were 13 perinatal deaths in T1DMP (9 still births, 4 neonatal deaths) giving a PNMR of 35/1000 births. There were 15 congenital abnormalities T1DMP (4 lethal congenital abnormalities). Corrected PNMR in T1DMP of 24/1000 compared to 6.0/1000 in the control population. C-section rate was 39% in T1DMP compared with 19.5%. The rate of pre term delivery (pre to 37 weeks) was 7.7% compared to 5.4%.The incidence of foetal macrosomia (Birth weight > 4.0 kg) was 23% compared to 15% in the control. Incidence of (Birth weight > 4.5 kg) was 8% compared to 2.5%.
Conclusion: Intensive management of T1DMP in combined endocrine obstetric clinics using complex (basal / bolus) insulin regimes result in improved perinatal outcomes over those previously reported. However outcomes have not yet reached those of non-diabetic population.

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A retrospective analysis on the outcome of pregnancy complicated by various degree of impaired glucose tolerance

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Background and Aims: Gestational Diabetes Mellitus (GDM) complicates 5–10% of pregnancies and determines an increase of maternal and fetal morbilities. Aims of our study were to evaluate the outcomes of pregnancies complicated by various degree of impaired glucose tolerance, diagnosed and followed in our Center from 1987 to 2002; furtheremore we wont to verify if the structured approach set up in our Center from the year 1998 could modify these outcomes.
Materials and Methods: We evalued 2737 pregnant women, of which 36,7% affected by GDM, 15.5% by G-IGT (Gestational Impaired Glucose Tolerance) and 47.8% with NGT (Normal Glucose Tolerance). Women have been subjected to glucose challenge test (GCT) between 24 and 28 gestational week (g.w.) or before (14–18 g.w.) when GDM risk factors were present. In presence of positive GCT (140 mg/dl), an OGTT(100g) was performed and interpreted according to Carpenter and Coustan criteria; the diagnosis of G-IGT was placed when one value was higher or equal to the normal range. Age, prepregnancy BMI, weight gain during pregnancy, g.w. at diagnosis, plasma glucose levels (fasting and 1 h after meal), HbA_1c, microalbuminuria were evaluated. As for maternal and fetal outcomes: timing and mode of delivery, maternal and fetal morbilities were taken into consideration. From 1998 the structured approach set up in our Center means that all pregnant women are followed, every two weeks, by an expert team composed by a diabetologist,a dietician and a nurse.
Results: Age and BMI were significantly higher in GDM than in G-IGT and NGT women ($p>0.000$). The weight gain was lower in GDM than G-IGT and NGT ($p>0.000$). Mean values of plasma glucose and HbA1c were higher in GDM with respect to NGT ones. 18.6% of GDM required insulin therapy. As for risk factors: age, family history for diabetes, obesity and previous GDM were posititely related to GDM diagnosis.When considering the frequency of GDM risk factor, we found a significantly increase of age and decrese of BMI in women followed in 98–02 with respect to those followed in 89–97. GDM patients delivered early; preterm delivery rate was higher in GDM than in G-IGT and NGT ones (10.9% vs 6.7% vs 7.2%) ($p>0,002$ GDM vs NGT). The rate of cesarean section was higher in GDM than G-IGT and NGT ones (42% vs 28,5% vs 28,1%) ($p>0.000$). Gestational hypertension was related to GDM. The rate of LGA ($p>0.002$) and hypoglycaemias in the GDM newborn was high.Both LGA and macrosomia were reduced in newborn of GDM mothers followed in 98–02 with respect to those followed in 87–97. At follow up only 33% of the GDM and 9% of the G-IGT were evalued. In these women the frequency of type 2 diabetes (3.6% of GDM and 7,9 of the G-IGT) and of IGT (8,2 of GDM and 2.6% of the G-IGT)
Conclusion: So, various degree of impaired glucose tolerance during pregnancy, even today, negatively affect maternal and fetal outcome;these alterations are also a risk factor for future impaired glucose tolerance development. Finally a structured approach and monitoring of diabetes in pregnancy could be usefull to reduce maternal and fetal complications.

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Continuous subcutaneous insulin infusion (CSII) versus multiple daily injection regimes using soluble human insulin or rapid acting insulin analoque in T1DM in pregnancy (T1DMP)

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Background and Aims: The benefit of using CSII compared to MDI insulin regimes in T1DMP remains uncertain.

Materials and Methods:Data on 43 singleton TIDMP, delivered after 37 weeks attending a combined DM/Obstetrical Service in Three University Maternity Hospitals. T1DMP subjects had HbA_1c measured at booking and monthly. All performed 7 home blood glucose tests (HBGM) daily, pre and 90 minutes post meals and bedtime. Data on one week of HBGM at 14 weeks, 26 weeks and 36 weeks were analysed as an index of day to day control in each trimester. MDI regimes involved use of SHI or RAA pre meals and NPH insulin given up to 4 times daily as basal insulin
Results: Subjects were in three groups; CSII Group, n=7, Soluble insulin (Actrapid®) Group (SHI), n=18 and Analogue Group (Lispro), (RAA), n=18. Baseline demographic data (Age, weight, diabetic duration and booking gestation) did not differ between groups. Hba1c was lower in CSII compared to SHI at booking (6.5 ± 0.6% Vs 8.4 ± 1.6%, $p<0.001$) and at 14

weeks (6.0 ± 0.8% Vs 7.0 ± 0.9%, p<0.01), 26 weeks (5.5 ± 0.7% Vs 6.5 ± 0.8%, p<0.01) and 36 weeks (5.7 ± 0.6% Vs 6.6 ± 0.7%, p<0.05). HBA1c was lower in CSII compared to RAA, but the difference was not statistically significant. Premeal HBGM were lower in CSII compared to SHI at 14 weeks (0.05<p<0.01). Percentage of HBGM readings <2.0 mmol/l was higher in CSII compared to SHI and RAA at 14 weeks (2.6% vs 0.8% vs 1.0%) and 26 weeks (0.8% vs 0.3% vs 0.5%) and 36 weeks (0.8% Vs 0.3% Vs 0.5%). Total daily insulin dose was lower in CSII at 14 weeks (41 ± 10 u/day) compared to SHI (65 ± 30 u/day,p<0.01) and RAA (60 ± 21 u/day), p<0.05) and 26 weeks (52 ± 13u/day) Vs (83 ± 40 u/day, p<0.05) and (75 ± 25 u/day, p<0.05). Maternal weight at delivery was similar between the groups. Birth weight did not differ in CSII (3.6 ± 0.6 Kg) Vs SHI (3.7 ± 0.7 kg) Vs RAA(3.7 ± 0.7 kg).

Conclusion: In this non-randomised study of intensively treated T1DMP use of CSII was associated with lower HbA_1c at booking and through pregnancy compared to MDI with soluble human insulin but not MDI using a rapid acting insulin analogue. Daily insulin dose was lower in CSII compared to MDI regimes up to 26 weeks gestation. Rates of hypoglycaemia (<2.0 mmol/l) may be increased in CSII. However these differences between CSII and MDI regimes did not appear to impact on birth weight in intensively treated T1DMP.

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Relationship between the infants' birth weight and glucose level fluctuations in pregnant patients with type 1 diabetes

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Background and Aims: It is generally believed that glycemic targets in diabetic pregnancies should mimic those found in normal pregnancies and treatment of type 1 diabetes should be aimed at producing a metabolic state such that the fetus does not recognize its mother as diabetic. The aim of the present work was to reveal correlation between HbA_1c, postprandial (PG), fasting (FG), mean blood glucose (MG) and hypoglycemia (HG) in pregnant patients with type 1 diabetes (T1DM), and their infants' birth weight (BW).

Materials and Methods: Totally, 102 pregnant women with T1DM were enrolled in the study (mean age – 26 ± 7yrs; diabetes duration – 10.2 ± 5.4yrs). Patients with nephropathy were excluded. Strict metabolic control was maintained and fetal surveillance was performed throughout the pregnancy. Thus, data obtained for the 3rd trimester (tr.), such as home-blood glucose monitoring (five-point profiles) and frequency of hypoglycemia episodes were enrolled in this work. The women delivered at 38–40 week of gestation. For statistic analysis Biostatistic Program was used.

Results: Analysis of data obtained for 102 patients revealed strong correlation between BW (3 760 ± 591.5 g) and PG (130.6 ± 32.9 mg/dl, r=0.82, P=0.000); BW and MG (121.3 ± 22.8 mg/dl, r=0.797, P=0.000); BW and HG frequency (r=0.780, P= 0.000); BW and FG (113.6 ± 23.6 mg/dl, r=0.709, P=0.000). Weaker correlation was observed between BW and HbA1c (6.33 ± 1.35%, r=0.623, P=0.004). In 21(20.5%) out of 102 newborns macrosomia was observed (BW -4 318 ± 314.3 g), in remaining 81 newborns BW was 3 383 ± 258.3g. In macrosomic patients HbA1c (7.18 ± 1.38%) and PG (162.8 ± 17.7 mg/dl) levels were statistically higher than in non-macrosomic ones (P= 0.003; P=0.000, respectively). In 28.5% of non-macrosomic patients HbA1c levels were below 6%, though frequency of HG episodes was higher in this group (13–19 episodes/month). In macrosomic patients strong correlation between BW and PG (110.6 ± 17.4 mg/dl, r=0.883, P=0.000), BW and MG (107.8 ± 14.1 mg/dl, r=0.708, P=0.012), BW and HbA1c (5.67 ± 0.7%, r=0.792, P=0.003), was observed, while correlation between BW and FG (101.7 ± 15.8 mg/dl, r=0.684, P=0.016) was much weaker.

Conclusion: Infants' birth weight strongly correlated with postprandial glycemia and mean glycemia in pregnant patients with T1DM throughout the 3tr trimester. While HbA_1c and fasting glycemia not always indicate to macrosomia development.

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Metabolic control, maternal and perinatal outcomes in type 1 diabetic pregnancies intensively treated with conventional insulin therapy vs. continuous subcutaneous insulin infusion

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Background and Aims: In order to approximate the outcome of diabetic pregnancy to that of non-diabetic population, maintaining glycaemia within normal levels still represents an important challenge. Because there is scarce information concerning the results obtained using continuous subcutaneous insulin infusion (CSII) in pregestational type 1 diabetes (T1D), our study aimed to compare pregnancy outcomes in T1D women treated with multiple doses conventional insulin therapy (CIT) with those receiving CSII.

Materials and Methods: We evaluated metabolic control, maternal and perinatal outcomes of 205 T1D pregnancies followed between 1998 and 2004. Thirty-six women were treated with CSII starting during pregnancy planning in order to obtain satisfactory metabolic control. The group treated with CIT included 169 women.

Results: There were not differences in clinical characteristics of both groups, including White's class (31.6 ± 4.3 vs. 32.5 ± 3.9 years of age; T1D duration 13.8 ± 8 vs. 11.9 ± 6.0 years, CIT and CSII, respectively). BMI was slightly lower in CIT (23.7 ± 3.2 kg/m^2) than in CSII (25.2 ± 2.8, p=0.006). HbA_{1c} immediately before conception was similar in both groups (6.8 ± 1.3 vs. 6.9 ± 1.3, CIT and CSII, respectively). Weight, insulin dose and metabolic control (3rd trimester HbA_{1c} 5.8 ± 1.0 vs. 6.0 ± 0.9) remained similar during the whole pregnancy. As regards maternal complications, foetal and perinatal outcomes, we did not observed any difference between women treated by CIT or CSII.

Conclusion: The results of our study indicate that in pregestational T1D women the use of CSII is associated with results in terms of metabolic control, maternal and perinatal outcomes during pregnancy similar to those obtained using multiple-doses of insulin in a conventional therapy.

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A study of the effect of glycaemic control and prepregnancy care on risk of pre-eclampsia in women with type 1 diabetes

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Background and Aims: Previous research has suggested that (in addition to nulliparity,) poor glycaemic control at the time of conception and early in the first trimester is associated with increased risk of pre-eclampsia in non-proteinuric type 1 diabetic pregnancy. We have examined the influences of glycaemic control throughout pregnancy and effect of prepregnancy care on risk of preeclampsia in women with type 1 diabetes.

Methods: A prospective cohort study of 290 consecutive non-selected type 1 diabetic pregnancies, 1991–2002. The relationship of pre-pregnancy care, parity, diabetes duration, age, presence of microvascular complications, weight, smoking and glycaemic control with risk of pre-eclampsia were examined. HbA_1c was measured at booking visit and then monthly. Preeclampsia was defined as 2 readings >140/90 with proteinuria of 300 mg/24 hours.

Results: There were 243 singleton deliveries. Pre-eclampsia developed in 31/243 pregnancies (12.8%). HbA_1c was significantly increased in women with preeclampsia compared to women without preeclampsia at 24 weeks (6.0% vs 5.6%, p=0.017) and also at 12 weeks (6.7%vs 6.2%, p=0.042). Using logistic regression, nulliparity was the strongest independent predictor (odds ratio, 3.5; p = 0.005). HbA_1c at 24 weeks gestation was the next strongest independent predictor (odds ratio, 1.65 for each 1% increase in HbA_1c; p = 0.01), although an association was also present with HbA_1c at 12 and 34 weeks. In contrast, HbA_1c at the booking visit was not independently predictive of pre-eclampsia. Prepregnancy care and other baseline variables tested were not predictive of preeclampsia.

Conclusion: The increased risk of poor glycaemic control to pre-eclampsia is not confined to the first trimester but persists throughout the second and early third trimesters also. There was no relationship between prepregnancy care and preeclampsia.

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Epidemiological and metabolic features of pregnant women with one abnormal glucose value during OGTT: a large cohort study

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Background and Aims: While the diagnosis of Gestational Diabetes (GDM) is based on two or more abnormal glucose values during a 100 gr OGTT, the meaning of a single abnormal test value remains a matter of debate. For this reason we have compared epidemiological and metabolic features characteristics of women with GDM with various OGTT plasma glucose profile.
Materials and Methods: A total of 3708 consecutive women with a positive glucose challange test (1 h>=140 mg/dl) underwent a 3-h 100 g OGTT. Based on Carpenter and Coustan's criteria, women were classified as GDM (>= 2 abnormal glucose values), Isolated Gestational Hyperglicaemia (IGH: one abnormal glucose value) and Normal Glucose Tolerance (NGT: no value exceeding).Antropometric parameters, blood pressure and lipid profile were collected in all women at the time of the OGTT (27 ± 3.2 gestational week).Moreover we have calculated HOMA-IR, insulinogenic (IS60: deltains60 min /deltagluc60 min) and insuilin index (IS-AUC: InsAUC/GlucAUC)
Results: GDM was diagnosed in 681 women (17,5%) while 707 had IGH (18,1%) and 2540 were NTG (64.5%). There was no difference in age, prepregnancy weight and BMI between GDM and IGH, though these parameters were all greater than in NGT (ANOVA p<0.01). Blood pressure, total, LDL and HDLcholesterol were similar in all groups, while triglycerides were higher in GDM (190 ± 44 mg/dl) and IGH (181 ± 53) than in NTG (162 ± 51; p<0.03). GDM and IGH had similar HOMA-IR (2.43 ± 1.8 and 2.26 ± 1.72) but they were more insulin resistant than NGT (NTG 1.98 ± 1.61; p<0.001). Similarly, impairment in insulin secretion was apparent in GDM (IS60 0.67 ± 2.3; IS-AUC 10.7 ± 6.3) and IGH (IS60 1.24 ± 1.4; IS-AUC 11.4 ± 5.8) as compared to NTG (IS60 1.43 ± 4.06; IS-AUC 12.5 ± 6.9; p<0.04 and p<0.0001 respectively). In MHG women the abnormal value was 26.7% at fasting (MHG1) and 73.3% (MHG2) post load. MHG1 showed HOMA IR and insulinogenic index significantly (p<0.05) higher than MHG2.
Conclusions: Retrospective survey indicates that the metabolic profile of pregnant women with isolated hyperglycemia is undistinguishable from that of women with GDM. These results highlight the need for determining to which extent isolated hyperglycemia may be sufficient to identy women at risk of complicated pregnancy and future development of type 2 diabetes.

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Reference range for HbA1c in pregnancy

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Background and Aims: HbA1c is an established retrospective marker for the assessment of glycemic control. Self monitoring of blood glucose and HbA_1c levels are the markers usually employed to evaluate also in pregnancy the glucose control. To reduce maternal and fetal complications ADA raccomends a level of HbA_1c <1% about the normal range.In thiscontext, few data are reported in literature about the reference ranges for HbA1c during pregnancy by using a DCCT aligned method. Therefore the aim of this study was to establish a HbA1c reference range using a DCCT-aligned HPLC method for pregnant women with normal glucose tolerance (NGT).
Materials and Methods: The study involved 5 Diabetic Care Units (Cagliari, Milano, Padova, Pisa). Fasting blood samples collected in EDTA tubes were obtained from 447 healthy caucasian pregnant women (NGT), and from 78 women with Gestational Diabetes (GDM) between the 11th and the 36th week of gestation. NGT had normal fasting plasma glucose and normal plasma glucose (< 140 mg/dL) 1 h after oral glucose challenge test (GCT). GDM was diagnosed according to Carpenter and Coustan criteria (OGTT with 100 g glucose). The HbA_1c was measured by an automated HPLC system (Menarini HA 8160) DCCT-aligned.using calibrators with a known title of HbA_1c (5.3–9.6%).
Results: The reproducibility of the HbA_1c measurements in the centers was good, the mean coefficient of variation being 3.1% (range 1.5–4.5%).
HbA1c in NGT women ranged between 3.3 to 5.7%, with 2.5–97.5 percentile confidence intervals of 4.0–5.5%. The median value was 4.8% in the period between the 19th to the 26th gestational weeks, and increased to 5.0% between the 28th to the 36th g.w.
No relathionships were found between HbA_1c and fasting and 1 h plasma glucose level in the GCT test. In GDM women the median value of HbA1c was 5.1%, being significantly higher than that of the NGT group (P<0.001).
Conclusion: We have established reference ranges for normal HbA1c during pregnancy with normal glucose tolerance. We think that these references could be useful to evaluate more accurately the glycemic control in pregnancy complicated by prepregnancy and gestational diabetes.

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Distribution of risk factors for gestational diabetes in the Austrian Gestational Diabetes Project (AGDP)

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Background: In Austria different tests are currently used for diagnosis of gestational diabetes (GDM) and there are divergent treatment strategies in different parts of the country. The frequency of GDM and the distribution of maternal risk factors in the Austrian population is not known.
Aim of the Study: To investigate the incidence of GDM and the risk profile in pregnant females by use of the diagnostic criteria proposed by the Austrian Diabetes Association (OGTT75g, 2 hrs: AGDP criteria: one abnormal plasma glucose value: fasting>95 mg/dl, 1 h>180 mg/dl, 2 h>155 mg/dl) in various Austrian hospitals (tertiary care centers) and to compare the detection rate of these criteria with the more simple WHO or the less strict ADA criteria.
Females and Methods: From January 2001 until April 2003 1479 pregnant women with at least one risk factor [> 30years; positive family history of DM2; ethnic group with high incidence of DM2; adiposity; history of impaired glucose metabolism, macrosomic newborns (> 4000g) or obstetric complications, hypertension or dyslipidemia; glucosuria, abnormal biometry] underwent an OGTT. In addition 171 women delivering at the respective hospitals but without any of these risk parameters were also investigated.
Results: 4.4% featured manifest diabetes according to WHO critieria, but no woman developed type 1 diabetes within the study period. 46% of the total group had GDM according to the AGDP criteria, while only 29% would be diagnosed as impaired according to WHO criteria and 14% according to ADA criteria. Even in the reference group 28% developed GDM compared to 45% in the group at risk (p<0.001). 91% of the women were white caucasians, 1.4% black-africans, 7.4% were of asian origin. GDMs had more often a history of GDM in previous pregnancies (11.2 vs. 3.5%, p<0.0001) or prediabetes (2.7 vs. 1.3%, p<0.04), LGA (11.8 vs. 6.3%, p<0.0002) or macrosomia (8.9 vs. 4.3%, p<0.001) by ultrasound or glucosuria (27,1 vs. 12.1%, p<0.0001) compared to women with normal glucose tolerance during pregnancy (NGT). Furthermore GDM were older (p<0.001) and more obese (p<0.0001) and had a higher weight gain in the first trimester (p<0.03) than NGT as well as a higher (p<0.009) frequency of first degree-relatives with type 2 diabetes. Asian origin, hypertension (p<0.003) and dyslipidemia (p<0.003) were also more common in GDMs. The risk to develop GDM was highest in case of glucosuria (OR 2.8), previous GDM (OR 2.6), adiposity (OR 2.1) or age higher than 30 yrs (OR 2.0), and in case of macrosomia (ultrasound) (OR 1.9).
Conclusion: This study shows i) that the AGDP criteria were more sensitive than those of the WHO, ii) a high rate of women with impaired glucose tolerance during pregnancy in Austrian hospitals both using the AGDP or the ADA criteria and iii) supports the demand of general screening for GDM in all pregnant women in Austria, but in particular in presence of any of these risk factors.
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Midpregnancy serum C-peptide concentration can predict later development of pregnancy induced hypertension in gestational diabetes mellitus

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Background and Aims: Insulin resistance and related hyperinsulinaemia have been associated with esential hypertension in nonpregnant individuals in different studies. Relatonship between increased C-peptide contrentrations and subsequent pregnancy-induced hypertension / PIH / have been found to be related by the authors already. the aim of the study was to investigate this relatonship in gestational diabetes mellitus / GDM /.
Materials and Methods: The 75 gram oral glucose tolerance test / OGTT / was performed according to World Health Organisation / WHO / criteria in 1395 discriminately pregnant women at 24–28 weeks of gestation between 01 August 2001 and 01 November 2003. Blood samples were collected after an overnight fast and 2 h after glucose ingestion. serum glucose and C-peptide concentrations / RIA,Biodata Rome, Italy / were measured in each sample. GDM was diagnosed in 108 of all pregnancyes / 9.9% / . Of the 108 participants 20 were excluded from analysis because of delivery in another place / n=15 /, preterm delivery before 36 weeks of gestation / n=3 / and prepregnancy or before the time of midpregnancy OGTT hypertension / n=2 / . Blood pressure was measured at the beginning and middle of pregnancy, and from 36 weeks of gestation weekly. PIH was defined as having systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg. Pre-eclampsai was defined as PIH in patients with proteinuria.
Results: Of the 87 GDM, 19 / 21.8% / developed PIH, including 3 who developed pre-eclampsia / 3.5% /. The fasting and 2 h C-peptide concdentrations among subjects developing subsequent PIH were 3.2 ± 1.3 / p< 0.005 / and 9.8 ± 3.0. The values were higher than those of the subjects who did not develop subsequent PIH: 2.2 ± 1.2 and 9.0 ± 2.7. Prepregnacy BMI of gravids was higher in PIH / 29.2 ± 5.3 p<0.0003 / than in no hypertension during gestation / 25.4 ± 5.3 /, thus the author examined the levels of C-peptide in the lower BMI / 25.7 ± 3.2 / group of PIH / n=10 /. The C-peptide concentration was significantly higher compaer to the normotensive group / 3.1 ± 1.6 p<0.03 / and level of C-peptide was moderately elevated / 9.1 ± 3.3 / .
Conclusion: The data document than high midpregnancy fasting and postprandial C-peptide concentrations are associated with subsequent development of PIH in GDM, and this association is independent of maternal weight, and the elevated midpregnancy C-peptide levles can predict the later develeopment of PIH.

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Morbidity and cognitive performance at time of conscript in 292 men whose mothers had pre-gestational diabetes compared with 870 healthy controls

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Background and Aims: Data on long term outcome in offspring of pregnancies with pre-gestational diabetes are scarce. We describe morbidity and cognitive performance in a cohort of young men exposed to maternal pre-gestational diabetes compared with non-exposed controls. The study is based on cohorts from two Danish regions (Rigshospitalet, Copenhagen and Aalborg Hospital, North Jutland County).
Materials and Methods: Evaluation at the military Draft Board is compulsory for all Danish men at the age of 18–19 years. The only option to escape examination at the Board is medically documented disease making them obviously unfit to service in which case the underlying diagnosis is filed. From hospital databases we identified a cohort of 296 boys (exposed) born from 1976 to 1984 of mothers with pre-gestational diabetes together with a control group of 870 men (non-exposed) matched at birth year and birth place. From the Conscript Registry we retrieved data obtained at medical examination on: 1) height, 2) weight, 3) cognitive performance (the Boerge Prien test being a correlate of the full scale intelligence quotient), and in cases of conditional acceptance or rejection from military service 3) the underlying medical diagnosis.
Results: Four babies with maternal diabetes died within the first month of life, one died before the age of 18 years, and thirteen had been granted postponement for examination leaving us with data on 278 survivors in the exposed and 870 in the non-exposed group. Data on main outcome variables are seen in the Table. The morbidity in terms of rejection for military service was significantly increased among exposed compared with non-exposed (RR: 1.26; 95% CI 1.02–1.51). There was no significant difference in individual categories of diagnosis leading to rejection. The exposed men were on average 4.2 kg heavier than non-exposed (p=0.00). There was a small difference in cognitive score of 1.4 units equivalent to 3.3 points at a standard full scale intelligence score (p=0.06).
Conclusion: Our data indicate a significantly higher body weight, a slightly higher morbidity, and a small difference in cognitive performance between the two groups. Further analyses incorporating e. g. data on gestational age and Apgar score are needed before firm conclusions regarding cognitive performance can be drawn.

	Exposed, n=278	Non-exposed, n=870	Signif. level
Rejected from service, no. (%)	147 (53%)	395 (45%)	RR: 1.26 (1.02–1.54)
Height, mean cm (SD)	180.3 (7.1)	180.3 (6.6)	p=0.96
Weight, mean kg (SD)	80.0 (15.9)	75.8 (14.0)	p=0.00
Body Mass Index, mean kg/m^2 (SD)	24.6 (4.4)	23.3 (4.0)	p=0.00
Cognitive score, mean (SD)	41.3 (9.5)	42.7 (9.6)	p=0.06

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Lower serum selenium concentrations are significantly correlated with inflammatory biomarker CRP values in gestational diabetic and control pregnant women

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Introduction: Selenium is an essential component in the antioxidant proteins glutathione peroxidase and thioredoxin reductase. It has also been shown to exhibit insulin-mimic properties in vitro and in vivo. A decrease in plasma selenium during pregnancy has been observed. Diabetic pregnancy is often complicated by a number of pathological conditions among which is increased oxidative stress. High sensitive C reactive protein (hsCRP) is an early indicator of increased lipid peroxidation.
Materials and methods: The serum selenium levels of gestational diabetic pregnant women (n: 20), non-diabetic pregnant women (n: 20) and healthy controls (n: 20) were compared, as well as their hsCRP values and lipid parameters. Blood was taken between the 24th and 28th week of pregnancy when the glucose load was performed. HsCRP was measured by immunturbidimetry. Selenium was determined via atomic absorption spectrometry following hydride generation.
Results: HsCRP values of gestational diabetics were significantly higher than in controls (8.9 ± 6.0 vs. 2.7 ± 3.7 mg/l, p<0.05). HsCRP values (6.3 ± 5.8 mg/l) of non-diabetic pregnant women were slightly higher than in controls but the difference was not significant. The serum selenium concentrations of gestational diabetics and non-diabetic pregnant women were 52.0 ± 10.7 and 40.5 ± 8.0 µg/l, respectively. Healthy controls had significantly higher serum selenium concentrations of 77.4 ± 14.8 µg/l (p<0.001 compared to both gestational diabetics and non-diabetic pregnant women). Cholesterol, HDL, LDG and triglyceride concentrations were significantly higher in pregnant women compared to controls with no difference between the two pregnant subgroups.
Conclusions: The higher hsCRP in gestational diabetic women may indicate an increased lipid peroxidation during pregnancy. Both gestational diabetic and non-diabetic pregnant women had significantly lower selenium concentrations than the healthy non-pregnant controls. The reduced selenium may contribute to increased lipid peroxidation in pregnancy. Our results raise the question of whether a small increase in selenium intake might help decrease oxidative stress in pregnant women, particularly in gestational diabetics.

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Insulin aspart vs. regular human insulin in basal/bolus therapy for patients with gestational diabetes mellitus: safety and efficacy

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Background and Aims: Pregnancy complicated by gestational diabetes mellitus (GDM) has been associated with increased risk of both maternal

and neonatal complications. Insulin aspart (IAsp) is a rapid-acting human insulin analogue designed to have faster subcutaneous absorption properties than regular human insulin (HI). The objective of this study was to compare the metabolic effects of IAsp vs. HI as the bolus component of basal-bolus s.c. regimens for patients with GDM.
Materials and Methods: In a single centre, randomized, parallel-group, two-arm, open-labelled trial, 27 women (age 29.7 ± 6.9 years) having GDM, HbA_{1c} <7% at diagnosis were randomized to receive either IAsp (5 min before meal) or HI (30 min before meal) using the NovoPen-3 injection device. Patients also received basal insulin (Novolin N). The trial period extended from the diagnosis of GDM (18–28 weeks) to 6-weeks postpartum.
Results: Mean reductions in HbA_{1C} values from baseline to 6 weeks post partum over time were not significantly different for the two treatment groups (Mean±SD: 0.3 ± 0.5% for IAsp vs. 0.1 ± 0.4% for HI). The most common adverse event was upper respiratory tract infection (14% IAsp, 23% HI). No major hypoglycaemic events were reported during the study. Specific antibodies to IAsp and HI as determined by radioimmunoassay remained relatively low (<1.5% binding of the specific antibodies). During gestation (36–38 weeks) average insulin-specific antibodies for IAsp and HI were 0.97% and 0.07%, respectively. Average percentile birth weights of the foetus for the IAsp and the HI groups were 40% and 44%, respectively. Overall safety profiles were similar for the IAsp and HI treatment groups.
Conclusion: The safety and effectiveness of IAsp was comparable to HI in pregnant women. The ease of use of IAsp injected just before meals rather than 30 min prior to meals may offer a more convenient therapy for management of diabetes for patients with GDM.
Support: Novo Nordisk Inc.

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Education and technology to improve glycaemic control

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Validation of automated insulin dosage recommendations made by HumaLink: a simulation study
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Background and Aims: HumaLink is a central computer that recommends insulin adjustments on the basis of the data from blood glucose (BG) self monitoring, sent in by telephone calls before meals. Patients receive advice concerning their premeal insulin doses.
Materials and Methods: To validate these recommendations, 14 patients were asked to send logbook pages, each recording data (4 BG /day, doses of ultrarapid analog and glargine) of 5 consecutive days. HumaLink was initialised with insulin doses intended to reach defined BG target and then fed with the BG data, with which it computed insulin dose recommendations. We obtained 1690 recommendations made by patients under real life conditions (data from logbook), HumaLink, 6 diabetologists and then retrospectively by the 14 patients themselves (blinded data).
Results: 1- Validation of HumaLink recommendations. For a given dose recommendation, we defined 3 pairs of intergroup comparisons between patients, physicians and HumaLink. For each group, as a mean, the percentage of full agreement (both members of the pair increase, do not change, or decrease) was superior to 59% and the percentage of full disagreement (patient or physician increase while HumaLink decreases and the opposite) was less than 4%. 2- Educational interest of HumaLink. Concerning the frequency of decisions, patients modify less often their insulin doses under real life conditions (70% of no change) than when they propose a posteriori their decisions (55%). This frequency is very similar to that observed with HumaLink (51% of no change). Diabetologists are the most aggressive (38%). To precise the "passive behavior" of patients under real life conditions, we showed that especially in this group, relative absence of insulin increase (HumaLink increase and patient doesn't change=76%) is more frequent than relative absence of insulin decrease (HumaLink decrease and patient doesn't change=24%). Moreover, by comparison to HumaLink, patients under real life conditions take more often decisions considered as less aggressive than HumaLink in the fight against hyperglycemia or aimed to avoid hypoglycemia (65%). The opposite was observed for diabetologists (44%). There was a significant positive correlation between the percentage of decisions less aggressive than HumaLink taken under real life conditions and HbA1c ($n=14$, $r=0.523$, $p<0.05$).
Conclusion: Under real life conditions, patients avoid especially decisions which may protect them against hyperglycemia (or which may lead to hypoglycemia). Following HumaLink advices could lead to a more active behavior of patients. These conclusions of this simulation study require further studies aimed to determine whether following HumaLink would improve metabolic control.

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Have we got it right? An interim evaluation of a user informed programme of education and support for people with type 1 diabetes
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Background and Aims: Very few education programmes have been developed in consultation with adults with type 1 diabetes, and therefore their understanding and personal experiences of diabetes have not been acknowledged. Current recommendations suggest that education programmes for people with type 1 diabetes are more likely to be effective if such individuals are actively involved in their development. It has been recommended that education programmes should focus on how to empower people with type 1 diabetes and that user-led and owned interventions are likely to be better placed to do this. This study was undertaken to assess the efficacy of a patient informed programme of education and support for people with type 1 diabetes, and evaluate improvements in psychological well-being and diabetes specific knowledge.

Materials and Methods: 97 people with type 1 diabetes recruited from North Bristol NHS Trust clinics participated in a 24 hour intervention (3 hours/week for 8 weeks). the mean age of participants was 43.6 years (sd=12.41) and had lived with their diabetes for a mean of 21.6 years (sd=12.41). 63% (n=61) of participants were female. The programme content was designed to allow interaction between participants and was facilitated by a Diabetes Nurse Specialist and a Clinical Psychologist. Core topics included: physiology of diabetes; managing hypoglycaemia, diet and alcohol; relaxation; management of stress and exercise; and the complications of diabetes. Diabetes specific quality of life scales (ADDQoL; PAIDS); measures of diabetes specific knowledge (ADKnowl); and psychological well-being (DES; HADS: CIDS) were completed by all participants pre- and post-programme.
Results: Data analysis has demonstrated a significant decrease in the number of perceived diabetes specific problems (PAIDS: t=3.956; df=32; p<0.0001); a significant increase in perceived empowerment (DES: t=4.373; df=32; p<0.0001); and individuals confidence in managing their diabetes (CIDS: t=5.252; df=32; p<0.0001).There were also improvements in diabetes specific knowledge relating to the effects of alcohol, management of hypoglycaemia and foot care. Descriptive statistics suggest improvements in perceived quality of life (ADDQoL).
Conclusion: This study has demonstrated that a patient informed programme of education and support for people with type 1 diabetes, can bring about significant improvements in psychological well-being, quality of life and diabetes specific knowledge. It has also confirmed that it is possible to empower people with type 1 diabetes through user led and owned interventions. Thus this study has demonstrated that a user informed programme of education and support for people with type 1 diabetes is an effective intervention. Furthermore, participants' increased knowledge and awareness following the programme has impacted on their perceived quality of life.
Support: Diabetes UK

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Impact of real-time telemedicine support on glucose self monitoring and blood glucose control in young adults with type 1 diabetes: a randomised controlled trial
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Background: Previous trials of telemedicine interventions in diabetes have not used automated transfer of data, real-time analysis and immediate feedback to the patient. Using such a system, for which full access to the patient's clinical measurements is also available to the carer, may lead to more effective use of telemedicine. We therefore set out to determine whether this approach, based on mobile phone technology, could improve glycaemic control for young adults with type 1 diabetes.
Materials and Methods: A 9-month randomised trial compared glucose self-monitoring (GSM) real-time result transmission by GPRS mobile phone with minimal feedback in a control group with graphical phone-based feedback and diabetes specialist nurse-initiated support using a web-based graphical analysis of GSM results in an intervention group. All patients aged 18 to 30 years with HbA1c levels of 8%–12% from a young adult diabetes clinic were eligible for inclusion.
Results: 93 patients (55 men) with mean diabetes duration of 12.1 years (SD 6.7) were recruited from a young adult clinic. There were 601 phone contacts initiated by nurses in the intervention group: an average of 13 per patient, with a mean duration of 7 min 9 sec, during which the nurse was able to discuss the patient's clinical measurements by accessing them on the patient's secure web page. During week 36 of the trial the median number of readings sent by the intervention group was 11 (IQR 1 to 28) compared to 0 (IQR 0 to 7) for the control group, Z=-3.5 P<0.0001). The median blood glucose level for the intervention group over the 9-month trial was 8.9 mmol/l (IQR 5.4 to 13.5) versus 10.3 (IQR 6.5 to 14.4) for the control group, P<0.0001). There was a significant reduction in HbA1c in the intervention group after 9 months from 9.2% (SD 1.1) to 8.6% (SD 1.4) (difference 0.6%, 95% CI 0.3 to 1.0, P=0.001) versus a smaller reduction in the control group from 9.3% (SD 1.5) to 8.9% (SD 1.4) (difference 0.4%, 95% CI 0.03 to 0.7, P=0.04), but the difference between the two groups at 9 months was not statistically significant. The proportion of patients achieving an HbA1c <= 8.0% at 9 months increased in the intervention group from 10.6% to 46.8% and in the control group from 19.6% to 23.9%, P<0.00001. The proportion of transmitted blood glucose readings <3 mmol/l was significantly higher in the intervention group compared to the control group 1650/29765, 5.3% versus 739/21400, 3.5%, P<0.0001. There was one recorded grade 3 hypoglycaemic episode in the control group.
Conclusion: Real-time feedback using GPRS mobile phone technology and targeted nurse support leads to sustained levels of monitoring, improvements in BG levels and attainment of HbA1c target. Use of this telemedicine system with nurse support may offer a cost-effective way to improve outcomes in routine practice without substantially increasing resources.
Support: The Vodafone Group Foundation provided an unrestricted grant for this work

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Searching for diabetes decision aids and related background information
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Background and Aims: The use of patient decision aids (DAs) is increasingly promoted to help patients make informed medical decisions consistent with their personal values. However, identification and quality assessment of DAs appears challenging. DAs are complex interventions and hence, evaluation of DAs needs access to background information about development processes, feasibility trials, and efficacy studies. So far, diabetes related DAs have not been systematically analysed. The aim of the present study was to identify diabetes related DAs and to investigate whether it is possible to find background information necessary for critical appraisal.
Materials and Methods: The 'Cochrane Inventory of Existing Patient Decision Aids' lists 331 currently available DAs verified according to the Cochrane definition of DAs. This inventory was used to identify DAs explicitly designed for patients with diabetes or relevant for diabetes care, in particular DAs on prevention and treatment of cardiovascular disease. DAs for anticoagulation therapy were not considered. In addition, a systematic search was performed in MEDLINE (PubMed) using the terms „Decision Aid*", „Patient Participation"[MeSH], „Decision Making"[MeSH], „Patient Education Handout"[Publication Type], and terms related to diabetes, diabetes care and cardiovascular disease. Furthermore, we continuously searched the World Wide Web for about one year. For tracking background information we systematically searched reference lists of the DAs, homepages of the authors and publishing groups, MEDLINE, PsycINFO, and the Cochrane-database; search terms additionally included the names of the authors and publishing groups, the full title and key parts of the DA.
Results: A total of 13 DAs relevant for diabetes care were identified. Only one DA specifically addressed patients with diabetes, the remaining referred to cardiovascular disease prevention and treatment in general (n=4) or treatment of single risk factors (hypertension, n=3; cholesterol, n=3; obesity, n=2); 12 DAs were listed in the Cochrane Inventory. Publications about development processes, feasibility trials, or efficacy studies were not derivable from the DAs' reference lists. The search for the background information yielded 1533 results in MEDLINE, 70 in PsycINFO, and 200 in the Cochrane-database. All titles and abstracts were screened leaving 27 publications for in-depth review, 6 of them referred to at least one of the 13 DAs. No additional background information was found on the homepages of the authors or publishing groups.
Conclusion: There are only few DAs relevant for diabetes care and it is difficult to identify them. It is impossible to find the background information necessary for critical appraisal of DAs. To facilitate testing of DAs by independent investigators we propose to create an electronic database which provides available DAs together with all relevant background information.

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Increased frequency of self-monitored blood glucose is associated with improvement in HbA1c in three treatment regimens: pump, SubQ, and non-insulin
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Background and Aims: How many BG's per day (BGpd) are necessary? Are there diminishing returns? Other studies have shown that increased BGpd improves HbA_1c. Linear regression models cannot adequately explore for a curvilinear effect which would be intuitively expected, i.e. a steeply declining curve for few tests and a diminishing slope for more tests.
Materials and Methods: We derived a non-linear mathematical model, $HbA_1c = C1 + C2 / (BGpd + C3)$. We reviewed 1280 charts of persons with diabetes from our large endocrine practice and fitted the results statistically to the model. Each of these data sets was divided into quintiles. T-tests were run between the 1st and 2nd versus the 4th and 5th quintiles.

Results: (see Tables) Each group is discussed in terms of how many BGpd are needed to achieve clinically meaningful A1c levels of 7.0% and 6.5%:
Pump Therapy: The curve is gradual. Benefits do not plateau until a high BG frequency. A mean HbA_1c of 7.0% is achieved with 5 BGpd, and 6.5% with 9 BGpd.
SubQ Insulin: Compared to Pump patients, SubQ patients usually do not test as frequently. Our graph shows robust benefits from more testing. At zero BGpd, HbA_1c is in the range of ten. With four BGpd, the SubQ curve drops steeply to match the Pump curve thereafter. SubQ patients achieve HbA_1c of 7 at 4 BGpd, and 6.5 at 8 BGpd. (On average, SubQ achieves clinical targets with fewer BGpd than Pump therapy, probably because more patients with DM2 are treated with SubQ insulin.)
Non-Insulin: Despite the absence of exogenous insulin's immediate BG-lowering effect, these patients benefit from more testing. The HbA_1c declines steeply from 7.6% to 6.6% in the 0–3 BGpd range, then plateaus. The response to more numerous BG's may include modification of exercise and diet and more aggresive use of oral agents. The curve and T-test show that this trend is highly significant, despite the admixture of numerous patients with endogenous insulin, who can maintain lower HbA_1c with few BGpd.
Conclusion: This non-linear model shows statistically significant effects for each regimen and supports increasing SMBG, with a rule of thumb of monitoring four or more BGpd if $HbA_1c > 7.0\%$.

	N	Formula	T-Test
Pumps	378	$HbA_1c = 5.65 + [10.3/(BGpd+2.84)]$	p<0.001
SubQ	278	$HbA_1c = 5.89 + [5.68/(BGpd+1.46)]$	p<0.001
Non-Insulin	552	$HbA_1c = 6.43 + [0.68/(BGpd+0.61)]$	p<0.001

	A1c Values From the Fitted Curves												
Pumps	9.3	8.3	7.8	7.4	7.2	**7.0**	6.8	6.7	6.6	**6.5**	6.5	6.4	6.3
SubQ	9.8	8.2	7.5	7.2	**7.0**	6.8	6.7	6.6	**6.5**	6.4	6.4	6.3	6.3
Non-Insulin	7.6	**7.0**	6.7	6.6	6.6	6.6	**6.5**	6.5	6.5	6.5	6.5	6.5	6.5
BGpd	0	1	2	3	4	5	6	7	8	9	10	11	12

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Impact of active vs usual algorithmic insulin titration and point-of-care vs laboratory measurement of HbA1c on glycemic control in patients initiating insulin glargine for type 2 diabetes: The GOAL A1C study
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Background and aims: GOAL A1C (Glycemic Optimization with Algorithms and Labs At Po1nt of Care) assessed the impact of active (weekly patient contact) vs usual (no unsolicited contact between visits) insulin titration monitoring and point-of-care (POC) vs laboratory glycosylated hemoglobin (HbA_1c) testing on glycemic control in patients with type 2 diabetes.
Materials and methods: This 24 week open-label study was conducted in 2164 mainly primary care sites and randomized 7893 patients with type 2 diabetes who were inadequately controlled on oral agents alone (HbA_1c >7%). Patients were randomized to either 1) usual insulin titration monitoring + laboratory HbA_1c testing; 2) usual titration monitoring + POC HbA_1c testing; 3) active insulin titration monitoring + lab HbA_1c; or 4) active titration monitoring + POC HbA_1c. Once daily insulin glargine (initiated at 10 U/d) was added to existing oral therapy and titrated up using a simple algorithm based on self-monitored fasting blood glucose (FBG).
Results: A total of 5721 patients completed the study. The mean age at study entry was 57 years and average diabetes duration was 8.4 years. The majority of patients were Caucasian (68%), followed by Black (16%), and Hispanic (10%), and most patients were overweight (average weight 98 kg). The main efficacy and safety endpoints are summarized in the table:

	Usual-Lab (n=1491)	Usual-POC (n=1363)	Active-Lab (n=1501)	Active-POC (n=1366)
Patients with A1C<7% at endpoint, %	31.4	29.5	36.4	40.6
HbA_1c, %*				
Baseline (BL)	8.8 ± 1.5	8.9 ± 1.6	8.9 ± 1.6	8.8 ± 1.5
Change from BL	-1.3 ± 1.5‡	-1.3 ± 1.6‡	-1.5 ± 1.6‡	-1.5 ± 1.5‡
FBG, mg/dL*				
BL	210 ± 69	214 ± 74	213 ± 74	210 ± 69
Change from BL	-77 ± 75‡	-81 ± 80‡	-89 ± 83‡	-87 ± 75‡
Glargine dose at 24 wks, U/d*	50 ± 35	50 ± 36	56 ± 36	55 ± 36
Weight change, kg	1.9 ± 7.4‡	1.6 ± 7.3‡	2.0 ± 7.0‡	2.0 ± 7.7‡
Hypoglycemia§				
BG<70 mg/dL‖	3.7	3.7	6.0	6.1
Severe¶	0.09	0.08	0.05	0.13

*Values = mean±SD; $P<.05$ vs Active-Lab; ‡$P<.0001$, BL vs end of study; §events per exposure year; ‖$P<.0001$, usual vs active; ¶event requiring assistance of another party

Conclusions: In a mainly primary care setting, significant HbA_1c and FBG reductions were achieved in all patient groups with the addition of insulin glargine (using a simple titration algorithm) to oral therapy. Active titration monitoring resulted in significant incremental reductions in HbA_1c (0.2%; $P<.0001$) and FBG (9 mg/dL; $P<.0001$) compared with usual titration monitoring. POC HbA_1c testing provided additional benefits over laboratory HbA_1c testing in the proportion of patients who achieved A1C<7%. Overall hypoglycemic rates were low.
This study was supported by Aventis Pharmaceuticals, a member of the sanofi-aventis Group

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Effects of a new concept of patient education with empowerment in type 2 diabetes
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Background and Aims: We have introduced a new concept of patient education based on the empowerment vision in type 2 diabetes. We report the results after 3 months from a 12 months intervention.
Materials and Methods: Eighty-one male (aged 61 ± 9 (mean±SD) years) and 71 female (aged 63 ± 8 years) type 2 diabetes patients treated in general practice were included in the study. The patient education was carried out by a mobile team in the local communities in a Danish county. The intervention consisted of 3 periods of education during 12 months: One week (26 hours) at baseline, 2 days (16 hours) after 3 months, and one day (6 hours) after 12 months. The education was based on the empowerment vision and delivered by physician, nurse, dietician, physiotherapist in groups of 10–12 patients. Bodyweight, HbA_1c, blood pressure, and self-rated health and physical activity (questions from The Danish Health and Morbidity Survey 2000 questionnaire) were outcome measures.
Results: Bodyweight decreased from 89.7 ± 18.4 to 88.0 ± 18.1 kg., p<0.001 and HbA_1c from 7.6 ± 1.3 to 7.1 ± 1.1%, p=0.001. There was no change in blood pressure (from 140.9 ± 15.3/82.2 ± 8.5 to 141.7 ± 16.0/81.8 ± 8.5 mmHg). Self-rated health increased ('overall health' p<0.001 and 'well-being' p=0.042). In the questionnaire, 39% of the patients reported increased physical activity but there was no overall change in the test of physical activity (p=0.119).
Conclusion: The first part of an education program with empowerment in a group setting with mobile education units was associated with improved glycaemic control, decreased bodyweight, and improvements in self-rated health in type 2 diabetes patients.

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Effects of a structured education programme on psychosocial and biomedical outcomes in newly diagnosed type 2 diabetes: results from the DESMOND pilot study

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Background and Aims: The UK National Institute for Clinical Excellence (NICE) has stated that individuals newly diagnosed with diabetes should receive self-management education. However, there is little evidence to inform on the quantity, content or process of this education. Therefore the DESMOND group (Diabetes Education and Self Management for Ongoing and Newly Diagnosed), a multidisciplinary collaborative, was set up to develop an effective self-management programme for individuals newly diagnosed with type 2 diabetes. This abstract reports the 3 month follow-up data from the pilot evaluation programme.
Materials and Methods: Individuals in 12 Primary Care Trusts across England attended a DESMOND workshop for patients newly diagnosed with type 2 diabetes (attendance within 8 weeks of diagnosis). They completed questionnaire booklets and biomedical data were collected at baseline and 3 months follow-up. Quality of life was assessed using the WHOQOL-BREF, illness beliefs using the IPQ-R, physical activity using the IPAQ and depression using the HADS.
Results: Of 226 people who completed baseline measures, 149 individuals (66%) completed 3 month follow-up questionnaires. Analysis using paired t-tests, showed that individuals were more likely at 3 months to agree that they understood their diabetes ($t = -7.92$; $df = 99$; $p < 0.001$), that it is a serious health risk ($t = 2.89$; $df = 99$; $p < 0.005$), that it is a chronic illness ($t = 2.09$; $df = 99$; $p < 0.05$) and that they can affect the course of their diabetes ($t = 1.9$; $df = 99$; $p < 0.05$). Physical quality of life improved from baseline to 3 months ($t = 2.0$; $df = 131$; $p = .022$) but there was no significant difference in the psychological, social and environmental domains of quality of life. Individuals reported doing vigorous activity on more days (mean increase = 0.5 days/week; $p < 0.05$), moderately intense activity on more days (mean increase = 0.6 days /week; $p < 0.01$) and walking on more days (mean increase = 0.5 days/week; $p < 0.05$). There were significant improvements in HbA_1c (baseline 8.5% SD 2.7; 3 month 6.9% SD 1.0; $p < 0.001$), systolic BP (baseline 146 mmHg SD 21; 3 months 135 mmHg SD 17; $p < 0.001$), diastolic BP (baseline 84 mmHg SD 11; 3 months 79 mmHg SD 9; $p < 0.001$) and body weight (baseline 83 kg SD 17; 3 months 80 kg SD 17; $p < 0.001$). The more an individual believes they can control their diabetes at 3 months, the greater their change in HbA_1c ($r = 0.24$; $p = 0.05$) and the less impact diabetes is seen to have on day to day life, the greater their change in HbA_1c ($r = 0.35$; $p = 0.006$). Individuals reporting a greater understanding of their diabetes reported more physical activity at follow-up ($r = 0.26$; $p < 0.01$).
Conclusions: This pilot data indicates that the DESMOND course for newly diagnosed individuals is changing important illness beliefs, and that these changes are related to improved outcomes. This clearly supports the need to evaluate the programme with an appropriately powered RCT.
Support: Diabetes UK and Department of Health

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i-DREAM: Interactive Diabetes Research Evidence Application in Management

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Background and Aims: Recently many randomized clinical trials which provide an evidence-base to reduce morbidity and mortality in patients with diabetes have been published. A major barrier to improving diabetes care is the implementation of research evidence into clinical practice. i-DREAM is a computer based active and interactive clinical tool that helps clinicians to make evidence-based decisions and provides a comprehensive diagnosis and management plan based on individual clinical parameters. The i-DREAM program identifies the relevant studies given the patient parameters such as blood pressure, cholesterol and HbA_1c % levels. This learner centred computer program also has links to abstracts and slide presentations of various diabetes clinical trials and hospital guidelines.
Materials and Methods: Clinical application of i-DREAM was explored in a trial. Fifteen clinicians of the diabetes team (11 doctors, 3 specialist nurses and 1 pharmacist) were given 10 case notes containing relevant patient details including biochemical parameters and asked to comment upon their clinical management. The i-DREAM program was then demonstrated and helped the clinician identify the relevant studies given the patient parameters such as blood pressure, cholesterol and HbA_1c % levels. The accuracy of their management plan was assessed using a scoring system based on identification of the abnormality, choosing the relevant study and applying its management plan(1 point each). The accuracy of the management plan was assessed based on the percentage scores before and after using the i-DREAM.
Results: On average, clinicians were aware only of '7.6' of the 12 trials. They used only using only '5.5' of these in clinical management management plan before using the i-DREAM. The mean score, based on identification of relevant studies and correct management plan, was 69% ranging from 0 to 87 before using i-DREAM. The score improved significantly to 98% now ranging between 97 and 100 ($p<0.001$) after using the tool.
Conclusion: i-DREAM is a simple tool that can be applied in clinical practice to use the best evidence from clinical trials for each individual patient according to their clinical characteristics. It is a tool that can ease clinical decision by combining patient values, clinical skills and best research based recommendation. It can also serve to educate patients thereby increasing their concordance to treatment. It can serve as a tool to educate the multiprofessional diabetes care team about the relevant research in diabetes care thereby helping in achieving comparable quality of diabetes care amongst various teams involved in it It is a software to ensure more patients have evidence based care.

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Potential mediators of the relationship between diabetic complications and psychological well-being

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Background and aims: To examine the relationship between diabetic complications and psychological well-being, and to determine whether health beliefs/perceptions mediate this relationship.
Materials and methods: The data were collected as part of the Diabetes Attitudes, Wishes, and Needs (DAWN) study, one component of which was a cross-sectional study of 5104 adults with type 1 or type 2 diabetes. There were roughly equal numbers of participants from 11 regions comprising 13 countries in Asia, Australia, Europe and North America. All data were self-reports, obtained through telephone or in-person interviews. The measure of macrovascular complications was presence of cardiovascular disease. The measure of microvascular complications was a count of several conditions: retinopathy, neuropathy, distal nephropathy, other neuropathy, and foot ulcers. Health beliefs/perceptions comprised 3 single-item measures: concerns about fulfilling future family responsibilities, concerns about one's financial future, and rating of one's general health. Psychological well-being was measured by: Positive psychological well-being (the WHO-5 instrument (5 items, alpha = .83), a measure of diabetes-related distress (4 items, alpha = .70), and a report of anxiety symptoms (2 items, alpha = .63). Multiple regression analysis of psychological well-being controlled for gender, age, education, type of diabetes, and duration of diabetes.
Results: Diabetes complications were common (18% with macrovascular disease; 50% with microvascular disease, 20% more than one microvascular disease). Both types of complications were independently associated with worse psychological well-being and together accounted for significant variance in WHO-5 (4%), diabetes-related distress (10%), and anxiety symptoms (10%). Microvascular complications accounted for more variance overall, in part because some respondents had multiple microvascular complications and there was a dose-response relationship between psychological well-being and number of microvascular complications. The presence of just 2 microvascular complications was associated with a clinically relevant reduction in psychological well-being (9 point reduction). Health beliefs/perceptions partially mediated the relationships between complications and well-being; degree of mediation was greater for macrovascular complications than for microvascular complications. Those with macrovascular and/or microvascular complications reported more family and financial concerns and worse general health. In turn, more concern and worse general health were significantly associated with poorer psychological well-being on all three measures.
Conclusions: The relationship of macrovascular and microvascular complications with poor psychological well-being is robust, manifesting itself across multiple measures and has clinical significance. Results suggest that this relationship is mediated partially by health beliefs and illness perceptions, i. e., complications increase concern about negative health and social consequences of diabetes, which in turn contributes to poor psychological well-being. Psychosocial interventions to increase psychological well-being might target these beliefs, e. g., attempt to reduce concerns about financial/family responsibilities by re-assessing how realistic these concerns are or identifying alternatives for managing these responsibilities.
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Prevalence of depression in diabetic patients in relationship with complications

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Background and Aims: To estimate the prevalence of depression considering metabolic control (A1c), diabetic complications and sociodemographic factors.
Materials and Methods: A total of 500 outpatients (BMI=29,51 ± 4,71; A1c:7,69 ± 1,69; men/women n= 255/245, respective mean age= 51,67 ± 10,68/49,27 ± 11,44) attending our diabetes center and 90 healthy subjects (men/women n= 41/49, mean age: 51,97 ± 11,05/49,83 ± 9,96) were assessed for symptoms of depression using Beck Depression Inventory (BDI). Scores between 13–24 were considered mild-moderate, >24 were considered as severe depression. The study and the control group were demografically matched. The patients were classified according to gender, educational and marital status, type of diabetes (T1DM n=83, T2DM n=417), BMI, A1c, and presence of diabetic complications. 340 patients had at least one diabetic complication and 160 were free of complications. We used chi-square and Mann Whitney-U tests to evaluate the prevalence of depression in all groups.
Results: The prevalence of depression showed difference in all groups (p=0,003). Patients who scored within the range of normal values were 49,4% of T1DM and 65% of T2DM. 32,5% of T1DM and 28,3% of T2DM patients had mild-moderate, 18,1% of T1DM patients and 6,7% of T2DM patients had severe depression. 71,1% of the control group scored normal, 22,2% had mild-moderate and 6,7% had severe depression. Older patients had significantly lower depression rates compared to the younger ones (p<0,001). Gender and educational status showed no significant effect on the prevalence of depression (resp. p=0,724 and p=0,263). The prevalence of depression increased parallel to duration of diabetes (p=0,002) and was significantly higher in patients with at least one complication compared to the patients without any complications. Higher HbA_1c levels indicated significantly higher prevalence of depression (p=0,001). No relationship between BMI and prevalence of depression (p=0,153) was found. Being single or divorced increased the risk of depression whereas being married indicated lower scores of depression (p=0,001). Although presence of nephropathy significantly increased depression in T1DM (p=0,048), retinopathy, neuropathy, diabetic foot, peripheric arterial disease and ischemic heart disease failed to show any significant correlation with depression (respectively p=0,337, p=0,057, p=0,278, p= 0,228, p=0,099). As to the type 2 diabetic patients all of the complications except nephropathy and diabetic foot increased prevalance of depression.
Conclusion: Diabetes mellitus increases the risk and severity of depression as well as other chronical diseases. Type and duration of diabetes, marital status, poor metabolic control and presence of diabetic complications are contributing factors especially in type 2 diabetics. The findings are going to be discussed further within the context of an ongoing cohort study.

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Increased risk for depression in patients with type 2 diabetes, but also in people with impaired glucose metabolism: the Hoorn Study

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Background and Aims: There is accumulating evidence that depression is more common in people with type 2 diabetes compared to the general population. However, few studies have investigated the prevalence of depression in people with impaired glucose metabolism. Besides, most prevalence-studies are uncontrolled and may also suffer from selection-bias, as they are conducted in specialized treatment settings. We studied the prevalence and risk factors of co-morbid depression in a community-based sample of older adults, comparing three groups: 1) type II diabetes patients 2) subjects with impaired glucose metabolism 3) normal glucose metabolism.
Materials and Methods: We used cross-sectional data from the population-based Hoorn Study, which included 1366 Dutch men and women aged 50–74 years. An OGTT was performed and depression was measured using the CES-D, a validated depression questionnaire. Moderate/severe depression was defined as a CES-D score > 15.
Results: The prevalence of pervasive depression was significantly increased in people with type 2 diabetes (21%) and subjects with impaired glucose metabolism (15%) compared with participants with normal glucose metabolism (8%). Regression analyses in the total sample yielded that older age, female gender, low education and having type 2 diabetes were the strongest predictors of higher levels of depression. A diagnosis of cardiovascular disease or neuropathy was also associated with increased symptoms of depression.
Conclusion: Results suggest that the prevalence of depression is not only increased in patients with type 2 diabetes, but also in people with impaired glucose metabolism. Functional limitations that often accompany co-morbid chronic disease may play an essential role in the development and maintenance of depression in type 2 diabetes.
Support: European Foundation for the Study of Diabetes (EFSD)-Novo Nordisk Programme

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Determinants of recovery from depression in type 2 diabetic patients after a one-year follow-up

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Background and aims: Little is known about the course of depressive symptoms in type 2 diabetic patients. This study was aimed at exploring changes in depressive symptoms after one year follow-up, and determining factors that may predict them.
Materials and methods: One hundred patients found to be at risk for depression by using CES-D scale in a random sample of 463 type patients, among them 30 diagnosed with clinical depression using structured clinical interview, were followed for one year. Problem areas in diabetes scale (PAID) and health-related quality of life measure (SF-12) were applied in addition to CES-D. Initially informed about their condition and treatment possibilities, the patients were contacted by phone at 3-month intervals, and re-assessed for depression after one year. They were aged 57 ± 7 yrs, with diabetes duration of 9 ± 6 yrs; 66% were female and 44% were on insulin therapy. Changes in depressive symptoms were determined using t-test. A reduction in depressive symptoms from CES-D≥16 to CES-D<16 was taken as being indicative of recovery, with multiple regression analysis used to determine its predictors.
Results: Fifty-six percent of patients with baseline CES-D scores ≥16 improved their depressive symptoms after one year and were below this cut-off (CES-D=9.8 ± 3.6), while 44% remained in the category indicative of pervasive depression (CES-D=27.3 ± 8.4). Regression summary for CES-D group (<16 vs. ≥16) as a dependent variable indicated that some demographic and psychological variables may predict recovery from depression (R=0.679 R_=0.461 F=4.432 p<0.00008). Social functioning as assessed by SF-12, type of depression, gender, the PAID score, physical functioning and age were shown to be the strongest individual predictors of depressive symptoms at one-year follow-up (Beta's were -0.48, 0.33, -0.30, 0.27, -0.300 and 0.25, respectively; all p<0.05). Patients more disturbed in social functioning, clinically depressed, male, of older age, with more diabetes-related emotional problems and worse physical functioning, were shown to have more difficulties in achieving recovery from depression.
Conclusion: The results suggest that some subgroups are more vulnerable to persistent depression and may need stronger support. Addressing diabetes-related emotional problems may be helpful in facilitating recovery from depression.
Support: Ministry of Science of the Republic of Croatia

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Glucose and lipid metabolism in individuals after failed suicide attempts

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Background and Aims: Recently, it has been suggested that insulin sensitivity markers (high plasma HDL cholesterol, low body weight, low systolic blood pressure) might be predictors of suicides and accidents. The study aim was to assess prospectively lipid and glucose metabolism in survivors of suicide attempts.
Materials and Methods: The study group comprised 183 subjects who survived suicide attempt (117 women, 66 men, mean [±SD] age 33.6 ± 12.4 years, body mass index BMI 24.3 ± 6.6 kg/m^2). Controls were 85 gender- and age- matched healthy persons (61 women, 24 men, mean age 35.4 ± 9.4 years, BMI 24.0 ± 2.4 kg/m^2). Biochemical, hormonal and psychological examinations were performed after the attempt. Suicidal attitude was assessed with MMPI subscale (Koss-Butcher Critical Positions Scale) and Beck's Self-Assessment Questionnaire. The examinations were repeated after 6 months in 67 persons.
Results: Results. Subjects after suicide attempts had significantly higher results in MMPI and Beck's tests than the controls: 10.4 ± 2.6 vs 3.4 ± 1.2 and 10.4 ± 2.3 vs 7.2 ± 2.4, p<0.0001, respectively). After 6 months the results of both tests were 5.6 ± 2.0 and 7.8 ± 1.7, respectively. Fasting plasma total cholesterol was significantly lower in the study group than in the controls (166 ± 35 vs 210 ± 43 mg/dl; p<0.0001) as were triglycerides (100 ± 51 vs 132 ± 72 mg/dl; p<0.0001) and LDL cholesterol LDL (99 ± 27 vs 119 ± 31 mg/dl; p<0.0001). After 6 months the respective values of plasma lipids in the study group were 196 ± 45, 118 ± 59 and 108 ± 31 mg/dl, respectively. Fasting plasma glucose was similar in the study and control groups (88 ± 8 and 89 ± 7 mg/dl, respectively), and it remained stable in the study group subjects after 6 months (88 ± 6 mg/dl).
Conclusion: Survivors of suicide attempts presented with low plasma total cholesterol, LDL cholesterol and triglycerides; however no differences in plasma glucose between these subjects and healthy controls were found.

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Is there any association between personality properties and glycemic control in patients with diabetes mellitus type 1?

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Background and Aims: The purpose of this study was to study association between personality properties and glycemic control in patients with Diabetes type I.
Materials and Methods: Two groups of patients with diabetes type I were studied (n=30 with HbA_1c <7%, and n=30 with HbA_1c >8%). A questionnaire with 209 questions relative to personality properties (extroversion, emotional stability, receptivity to experience, affability, neurotism) as given by Dpt. Psychology of UCL was given to the patients. Statistical analysis included x^2 t-test and logistic regression analysis.
Results: The mean age of patients was 26.1 ± 5.7 years, the mean duration of diabetes was 10.2 ± 6.8 years and 42% males and 58% females were examined. There was positive association between glycemic control and high cordiality (OR=5.68, p=0.0016), moderate compliance (OR=7.5, p=0.025), moderate imagination (OR=8.272, p=0.014). There was negative association between glycemic control and high stress (OR =0.177, p=0.028), high neurotism (OR= 0.025, p=0.031), moderate confidence (OR= 0.154, p=0.018) and moderate shyness (OR=0.188, p=0.015). Independently statically significant variables associated with glycemic control were moderate and high compliance (OR=209, p=0.017) and high stress (OR=0.009, p=0.005).
Conclusion: There is statistically significant association between glycemic control and some parts of personality such as stress and compliance. Physicians must know the parts of personality contributed to the glycemic control by positive or negative aspect.

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Alexithymia in subjects with type 1 diabetes

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Background and Aims: The main goal in treating subjects with diabetes is the prevention of associated disease conditions by optimisation of blood glucose control and the maintenance of a good quality of life. Both metabolic control and quality of life are influenced by several individual factors like personal attitude and psychopathological tracts such as alexithymia. This psychological construct, initially observed in subjects with psychosomatic and psychiatric disorders, is linked to failure in psychological self-regulation and deficit in experiencing emotional life. It has been previously shown that alexithymic features may interfere with the affected subject's disease-coping strategies. However, how different levels of alexithymia are reflected on the behaviour of disease affected subject, is still a matter of investigation.
In the present study we tested the hypothesis that subjects affected by type 1 diabetes with high level of alexithymia may be less able to control their glucose levels and to adhere to treatment mostly because their higher stress perception. Previous studies have shown that psychological stress in diabetes can lead to poor metabolic control in diabetic patients. The hypothesis is that alexithymic subjects have higher stress levels because their difficulty in regulating emotions and impaired ability to monitor signals from the body.
To verify this hypothesis we investigated the relationship between alexithymia and stress perception in subjects with type 1 diabetes.
Materials and Methods: We studied 50 consecutive subjects affected by type 1 diabetes (mean age 29 years) attending one single out patient diabetes clinic in an University based hospital. Alexithymia was assessed with a validated translated version of TAS-20 (Bagby 1994), a self-report measure of the alexithymia construct. Alexithymia was used as a continuous variable. Perceived stress was assessed by Perceived Stress Scale (PSS), a 14-item self report tool used to provide a global measure of perceived stress in daily life.
Results: According to standard evaluation parameters (score >51), alexithymia was detected in 24 (48%) of diabetic subjects. Data was then evaluated separately depending on the level of alexithymia. The group of sub-

jects with high level of alexithymia (n=24) showed mean TAS values of 55.24 ± 3.4 and PSS of 28.6 ± 5.44; the group of diabetic subjects with low level of alexithymia (n= 26) scored 33.48 ± 4.32 for TAS values and 19.88 ± 5.13 for PSS. Such differences were statistically significant (p<0.0001, Bonferroni test,). A positive correlation was also found between TAS and PSS (linear regression r = 0.69, p< 0.0001).
Conclusion: We demonstrated that a clear relationship exists between the alexithymic construct and perceived stress in type 1 diabetes supporting our initial hypothesis. Since difficulty in maintaining optimal glucose control has been previously shown to be influenced by alexithymia and stress, it can be inferred that impairment in disease effective management in presence of alexithymic subjects is due to the co-occurrence of stress perception.
Support: Università Campus Bio-Medico / Roche Diagnostics

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The illness beliefs of people newly diagnosed with type 2 diabetes: results from the DESMOND pilot study
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Background and Aims: DESMOND (Diabetes Education and Self Management for Ongoing and Newly Diagnosed), a multi-disciplinary collaborative, responded to the UK National Service Framework's standard for structured education in routine care by producing a self-management education programme for individuals newly diagnosed with type 2 diabetes.There are theoretical and empirical reasons to suggest a relationship between illness beliefs and both self-management behaviours and biomedical outcomes. Therefore a core theoretical model in DESMOND is that education must start with the patient's existing belief systems in order to facilitate new learning. Low mood may also impact on the learning process and there is growing evidence as to the importance of low mood or depression in predicting poor biomedical outcomes in diabetes. This abstract reports the illness beliefs of patients newly diagnosed with type 2 diabetes, and their relationship to depressive symptomatology. The data are from a pilot evaluation of the DESMOND programme.
Materials and Methods: Patients newly diagnosed with type 2 diabetes from 12 Primary Care Trusts across England were invited to attend a DESMOND education programme. Before attending the course patients completed a questionnaire booklet containing scales from the Diabetes Illness Representations Questionnaire, and the Hospital Anxiety and Depression Scale.
Results: Questionnaires were completed by 226 patients of whom 95% were Caucasian, 49% were male and the mean age was 63 years. 66% said they would have diabetes for the rest of their life, 23% said they had a mild form of diabetes, with 40% uncertain, and 5.5% agreed that diabetes shortens life. 19% scored on or above the borderline for depression (score 7) and 4% had high scores for depression (score 12 and above). Higher depression levels were associated with individuals who rated diabetes as having a greater impact on their daily life ($r = 0.275$; $p < 0.001$), did not understand their diabetes ($r = 0.21$; $p = 0.013$) and did not feel they could affect the course of their diabetes ($r = 0.218$; $p < 0.001$).
Conclusion: Clearly many patients do not understand diabetes and still think it is temporary or mild. Incorrect beliefs need to be elicited, if beliefs predictive of self-management, such as perception of threat which is avoidable, are to prevail. The finding that low mood is prevalent in this group is also important to acknowledge as it may impact on the education process. Analysis of follow-up data collected in the study will allow an exploration of whether the illness representations associated with mood are a determinant or a result of low mood induced cognitive processes.
Support: Diabetes UK and Department of Health

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Psychological barriers to insulin therapy in type 2 diabetics
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Background and Aims: Insulin therapy is an effective means to fight the complications of type 2 diabetes. In many cases, however, therapy outcomes are compromised by undue delay of therapy initiation. The purpose of our study was to estimate the prevalence and strength of psychological barriers to insulin therapy in German patients with type 2 diabetes.
Materials and Methods: We conducted a mailed survey of a nation-wide sample of general practitioners and internists and their outpatients with type 2 diabetes. Among others, the patient questionnaire covered the following topics: willingness to use insulin, perceived seriousness of type 2 diabetes, perceived susceptibility to diabetes complications, expectations regarding beneficial and adverse insulin effects, competence expectations, fear of injections and blood glucose tests, perceived incompatibility of insulin therapy with everyday routine, and social barriers imposed by family, colleagues, and others. Statistical analyses comprised description, multivariate exploration, and logistic regression analysis to identify factors determining a negative attitude towards insulin.
Results: 365 of the 729 patients interviewed expressed willingness to use insulin therapy (>5 on a 10-point rating scale). Of these, 40.5% were already treated with insulin, as opposed to 5.9% of those expressing greater reluctance to use insulin. Principal component analysis of the 35 rating questions revealed 7 factors explaining 60% of the total variation. Five factors (fear of injections, social barriers, aversion to dependency on insulin, negative competence expectations, fear of insulin-related adverse effects) were associated with reluctance to use insulin, two factors (positive outcome expectations, fear of the consequences of diabetes) correlated with a positive attitude. The most important predictor of patients' reluctance was their lack of positive outcome expectations.
Conclusion: Unwillingness to use insulin cannot be explained by fear of injections alone. Education programs for patients with type 2 diabetes should stress the beneficial effects of insulin therapy.
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The effect of intervention education of prevention the diabetic foot on the patients with diabetes
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Background and Aims: Diabetic foot, which can result in loss of limbs and death, is a major health problem for people with diabetes mellitus. The study was aimed to evaluate the effectiveness of prevention of diabetic foot by individual diabetic foot intervention education program from the diabetes education nurse.
Materials and Methods: A randomized controlled trial was conducted in the study. A total of 220 patients with diabetes with systematic sampling were assigned into 86 cases of intervention education group (n=110, missing 24 cases) and 92 cases of control group (n=110, missing 18 cases). The patients in the control group received the routine diabetic treatment and general diabetes education. The patients in the intervention education group received individual education of diabetic foot care from the diabetes education nurse in outpatient department besides the education contents of the control group. The effect was evaluated before education and nine months after education. The baseline assessment was no significantly difference between the education intervention group and the control group(P>0.05). Baseline data collection assessment included diabetes type, duration and treatment,smocking history, lower-limb physical examination, tests for sensory and neuropathic symptoms and macrovascular perfusion in the foot, metabolic items: such as FBG,PBG, HbA_1c BMI,blood pressure and foot care knowledge and behavior.
Results: The study showed that participant's reorganization and screening knowledge of diabetic foot,daily foot care knowledge and behavior,knowledge of choosing proper shoes and sock wears,the knowledge of cutting nails were significantly improved in the education intervention group after education(P<0.05). Foot problems such as Callus(51.16% vs 62.57%), fungal infection(10.47% vs 30.43%), foot lesions(10.47% vs 30.43%), dry and crack skin (43.02% vs 59.78%) were reduced significantly during the follow-up of nine months in the education intervention group(P<0.05-P<0.001). FBG, PBG, HbA_1c,BMI and blood pressure were controlled much better in the education intervention group, compared with the control group, the difference was significant found in the study(P<0.05).
Conclusion: Individual diabetic foot intervention education program practiced by the diabetes education nurse was feasible and effective. It was found that diabetic foot education program had a positive affact on improvement of foot care knowledge and behavior of patients and prevention the foot ulceration in the midum-long term.

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Diabetes in childhood

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Birth weight and elevated albumin/creatinine ratio in youth with diabetes: the SEARCH for Diabetes in Youth Study

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Background and Aims: Low birth weight is associated with an increased risk of several chronic conditions, including renal disease. Virtually no data exist on the relationship of birth weight (BWT) and renal disease in youths with diabetes. This relationship was explored using data from SEARCH for Diabetes in Youth, a U.S. multi-center, multi-ethnic study of youth with diabetes.
Material and Methods: SEARCH is a six-center, population-based surveillance effort ascertaining cases of diabetes mellitus in youth less than 20 years of age in the USA. A spot urine sample was obtained from 2633 subjects with diabetes (age 13.7 ± 4.5 years, 50% male, DM duration 5.1 ± 3.9 years) and albumin (Dade-Behring, BNII nephelometer) and creatinine (Jaffe method, Hitachi 917) were measured. Elevated albumin excretion was defined as an Albumin to Creatinine Ratio (ACR) ≥30 µg/mg (286 subjects with elevated ACR). BWT was categorized as low (<2500 grams), normal (2500–4500 grams), and high (≥ 4500 grams). The relationship between BWT and elevated ACR was explored using multiple logistic regression analysis.
Results: The prevalence of elevated ACR was 14.4% in youth with low BWT, 9.6% in those with normal BWT and 16.9% in those with high BWT. BWT category was significantly associated with elevated ACR (p=0.02), when adjusted for age, gender, race/ethnicity, DM duration, DM type, body mass index (BMI) and Hemoglobin A1c (HbA_1c). Elevated ACR was higher in youth with low BWT and high BWT than in subjects with normal BWT (Table), suggesting a U-shaped association between BWT and the prevalence of increased ACR. In the multivariate analysis, type 1 diabetes [0.29 (0.17–0.50)], DM duration <5 years [0.72 (0.52–1.00)], BMI z-score [0.83 (0.70–0.98)], female gender [1.60 [1.18–2.19)], and HbA_1c [1.23 (1.13–1.34) were significantly related to elevated ACR.
Conclusions: The pattern of the data suggests that a U-shaped relationship exists between birth weight and elevated urine albumin excretion among youth with DM. The reasons for this association are yet to be explored but may be different for each end of the birth weight spectrum.

Birth Weight Category as a Predictor of Albuminuria, Odds Ratios and 95% Confidence Intervals

Birth Weight Category	Odds Ratio	95% Confidence Interval
Low BTW	1.3	0.73–2.17
Nortmal BTW	Reference	
High BTW	2.6	1.30–5.00

SEARCH for Diabetes in Youth is funded by the Centers for Disease Control and Prevention (PA number 00097) and supported by the National Institute of Diabetes and Digestive and Kidney Diseases

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In childhood diabetes the increase in oxidative stress is not related to the severity of endothelial dysfunction

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Background and Aims: Diabetes mellitus is associated with both endothelial dysfunction and oxidative stress (OS). It is not known if these abnormalities are already present in childhood and if they are interrelated. The aim of this study was to investigate this question in children with T1DM.
Materials and Methods: Forty-one T1DM children and adolescents (age 9 to 21 years; 20 males; median duration DM 6 years; BMI 19.5 ± 2.8 kg/m^2) were matched for sex, age, length and weight with non-diabetic subjects (CO, n = 38). Flow-mediated dilatation (FMD), intima media thickness at the level of the carotid (IMT) and a fasting blood sample for the measurement of OSS and endothelial cell markers were taken in the outpatient clinic.
Results:DM children had lower hydrophilic plasma antioxidant capacity (154 ± 23 vs 188 ± 34 µmol/L Trolox equivalents in CO, p<0.0005) which was associated with lower levels of plasma ascorbate (67 ± 18 vs 81 ± 27 µmol/L in CO, p 0.011), protein thiols (4.14 ± 1.66 vs 5.17 ± 1.84 µmol SH/g protein, p 0.018), uric acid (3.45 ± 0.60 vs 4.20 ± 1.05 mg/dL in CO, p 0.002) and albumin (4.01 ± 0.34 vs 4.52 ± 0.23 g/dL in CO, p 0.002). Lipid peroxidation (malondialdehyde) was higher (0.59 ± 0.18 vs 0.49 ± 0.14 µmol/L in CO, p 0.005) in parallel to higher serum cholesterol and triglycerides. Serum ferritin was elevated in DM (40.8 ± 21.5 vs 31.8 ± 17.2 ng/mL in CO, p 0.046). Even though girls had higher lipid levels, the OS differences due to DM persisted after correcting for the effect of gender. Although homocystein was lower in DM (6.56 ± 1.4 vs 7.59 ± 2.0 µmol/L in CO, p 0.025) and E-selectin and endothelin did not differ, the von Willebrand factor was higher (148 ± 38 vs 122 ± 39% in CO, p 0.004) and ICAM tended to be higher (p 0.09). TNFα, fibrinogen and CRP did not differ in DM but were higher in girls (p 0.05, 0.014 and 0.03 respectively).
Although blood pressure and IMT did not differ, FMD was lower in DM (6.65 ± 1.88 versus 7.92 ± 1.60% in the CO, p 0.003; especially in boys, p 0.05 for the interaction between gender and DM). FMD was related inversely to ferritin (r -0.26, p 0.024), HbA_1c (r -0.29, p 0.013) and triglycerides (r -0.29, p 0.014) and positively with fibrinogen (r 0.28, p 0.023). FMD did not correlate with endothelial cell or OS markers. An inverse relation to IMT was only evident in DM (r -0.35, p 0.033).
Conclusion: Even at an early age diabetes mellitus is associated with both oxidative stress and impairment of endothelial vasodilatation. However, the severity of endothelial dysfunction is not explained by the degree of OS or the levels of endothelial markers in serum but by the alterations in IMT, serum lipids, HbA_1c and ferritin as well as by gender differences.

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Relationship between elevated hsCRP, lipid profile, and carotid artery intima media thickness in adolescents with type 1 diabetes mellitus

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Background: Atherosclerosis appears to begin in youth with T1DM in association with an abnormal lipid profile. Intima-media thickness (IMT) of the carotid artery has been used as a subclinical index of early atherosclerosis. hsCRP is an independent marker of cardiovascular disease (CVD) risk, but its role in adolescents with T1DM is unknown.
Aims: To quantify the presence of subclinical atherosclerosis by measuring IMT and to determine its association with hsCRP, serum total cholesterol (Chol), LDL, HDL, triglycerides (Tg), apolipoprotein B (apoB), and lipoprotein(a) (Lpa) in youth with T1DM.
Materials and Methods: Cross-sectional cohort study. IMT, hsCRP, and fasting lipid profiles were measured in 61 patients with T1DM, mean age 16.1 ± 2.76 yr, mean duration of diabetes 7.1 ± 4 yr, mean HbA_1c 8.3 ± 1.3%, and in 14 age-matched controls. According to AHA/CDC recommendations for CVD risk assessment, hsCRP was divided into 3 groups: <1.0 low CVD risk, 1.0–3.0 average CVD risk, and 3.1–10.0 high CVD risk. Rank sum and non-parametric Kruskall Wallis rank tests were used for statistical analysis

Results: Subjects with T1DM had significantly higher IMT compared to controls (0.562 vs 0.515, p=0.005). Results of between-group comparisons are presented in the Table.

Comparison of hsCRP groups predicting CVD risk to mean lipid levels and IMT

hsCRP mg/L	Chol mg/dL	LDL mg/dL	Tg mg/dL	apoB mg/dL	Lpa mg/dL	HDL mg/dL	IMT mm
Group 1 <1.0 (n= 27)	169.75	98.68	71.25	77.22	30.39	56.92	0.562
Group 2 1.0–3.0 (n= 15)	196.82	120.52	102.88	95.88	45.84	55.76	0.571
Group 3 >3.0 (n= 19)	192.78	110.13	155.43	95.61	38.21	52.04	0.555
p Comparison of 1 vs 2	0.012	0.022	0.008	0.012	0.399	0.933	0.462
p Comparison of 2 vs 3	0.459	0.239	0.359	0.945	0.787	0.218	0.314

Conclusion: Adolescents with T1DM have significantly higher IMT compared to healthy controls. Dyslipidemia is also present in adolescents with T1DM, as well as increased inflammatory activity shown by an increased concentrations of hsCRP. A hsCRP level ≥ 1.0 mg/L appears to be a critical cut point and significantly correlates with unfavorable alterations of Chol, LDL, Tg, and ApoB. However, there is no correlation between elevated hsCRP and IMT in our cohort. In summary, although hsCRP may be a useful marker to detect endothelial inflammation that clusters with dyslipidemia and contributes to increased CVD risk, it is not associated with early unfavorable changes in IMT in adolescents with T1DM.
Support: American Diabetes Association

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Prehypertension in children and adolescents with type 1 diabetes mellitus
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Background and Aims: The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents 2004 includes the new term - prehypertension, defines as average systolic blood pressure (SBP) or diastolic blood pressure (DBP) >= 120/80 mmHg (even the BP < 90th percentile) but < 95th percentile for gender, age, and height during at least three independent measurements. Prehypertension is an indication of increased risk for developing hypertension in future and requires therapeutic lifestyle changes. Hypertension and even elevated systemic BP is one of the most important risk factor of diabetic micro- and macroangiopathy. The aim of the study was to evaluate the prevalence of prehypertension and relationship between prehypertension, metabolic control and chronic complications in children and adolescents with type 1 diabetes mellitus.
Materials and Methods: 63 patients with type 1 diabetes mellitus (35 male), aged 12.2–18.5 years (mean - 16.4+/-1.97), with duration of diabetes 0.5–16.9 years (mean - 5.5+/-4.1), without evidence of arterial hypertension were recruited. In all patients 24-hour automatic blood pressure by oscillometric device was performed. The individuals with > 40% of SBP and/or DBP >= 95th percentile were defined as hypertensive, > 40% SBP and/or DBP >= 120/80 mmHg but <95th percentile – as prehypertensive, = 120/80 mmHg – as normotensive. Urinary albumin excretion rate was estimated by chemiluminescent enzyme immunoassay method. Ophthalmoscopic examination (fundus camera) and power spectral analysis of heart rate variation (commercial equipment and software) were performed. HbA_1c was examined by HPLC. Lipids levels were measured by an enzymatic method. Body mass index (BMI) and daily dose of insulin (DDI) were calculated.
Results: None of the study patients had hypertension; however, in 23 individuals (36.5%) prehypertension was diagnosed. In the table we compared clinical characteristics of the patients with normal and elevated BP.
Conclusions: 1) Prehypertension is common in children and adolescents with type 1diabetes mellitus. 2) The prevalence of prehypertension is associated with elder age and longer duration of diabetes. 3) In prehypertensive patients the shift of the sympathovagal balance toward sympathetic activation was found. 4) The tendency of greater BMI, higher daily insulin dose and elevated serum lipids levels was observed in prehypertensive patients.

	Normal blood pressure	Prehypertension	p
Age (years)	16+/-2.2	17.2+/-1.1	0.03
Sex (M/F)	20/20	15/8	0.24
Duration of diabetes (years)	4.7+/-3.4	7.1+/-2.1	0.04
DDI (U/kg)	0.77	0.88	0.06
HbA_1c (%)	7.5	7.7	0.37
BMI (SDS)	0.42	0.87	0.11
Cholesterol (mg/dl)	172	186	0.13
Triglycerides (mg/dl)	85	114	0.09
Microalbuminuria (n)	-	2	0.06
Retinopathy (n)	1	3	0.1
LF/HF ratio	0.79	1.02	0.02

Support: Medical University of Lodz Grant– 502-11-062

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Diabetic ketoacidosis at onset of diabetes in a representative sample of the US population
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Background and Aims: SEARCH for Diabetes in Youth is a multi-center registry of diabetes in individuals <20 yrs old in 5 million children representing wide ethnic and socioeconomic spectrum of the U.S. population. Our goal was to determine the prevalence and predictors of diabetic ketoacidosis (DKA) at onset of diabetes in this population.
Materials and Methods: In 2002, SEARCH identified 1,268 incident cases of physician-diagnosed diabetes; of those, 831 (66%) had onset medical records reviewed. DKA was defined by blood bicarbonate <15 mmol/l and/or pH<7.25 (<7.3 if arterial or capillary) or other medical records confirming DKA.
Results: Most of the cases (57%) were hospitalized at onset and an additional 12% were seen in an emergency room only. DKA was present in 23%. The prevalence of DKA decreased significantly with age from 36% in children <5 yrs of age to 16% in those >14 yrs, but did not differ significantly by gender or ethnicity. In univariate analyses, DKA was associated with lower income (p=0.008) and lower parental educational achievement (p=0.012) and more frequent in children with no medical insurance (33%), compared to those having indigent coverage (24%) or those with private insurance (13%). In multivariate logistic regression, adjusting for the effects of center and setting (hospital, ER), DKA was associated independently with younger age (OR=3.1 95%CI 1.2-7.5, for age-group <5 yrs vs. >14 yrs) and lower income (OR=3.7; 95%CI 1.5–9.0, for families with annual income < $35,000 vs. those with income $75,000–100,000).
Conclusion: One in four of U.S. youth with newly-diagnosed diabetes presents with DKA. Young and poor children are disproportionally more affected.
Support: SEARCH for Diabetes in Youth

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Metabolic control and interactions with diabetes-related knowledge, motivational problems and technical devices in children and adolescents with type 1 diabetes mellitus - DIDOM, an intervention trial
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Background and Aims: While the central role of HbA_1c for the prediction of diabetes-related complications in patients with type 1 diabetes is generally accepted, in children and adolescents the level of metabolic control achieved during routine care differ widely. In the pediatric age-group limited information is available on factors that influence metabolic control. Hence, it was the goal of DIDOM, an intervention trial, to identify parameters influencing metabolic control and to develop strategies to overcome the problems.
Materials and Methods: All patients (n=161) admitted to our hospital during of 10 months were included and underwent an extensive test battery (standardised analyses of self-management, handling of insulin injections,

self-monitoring, diabetes-related knowledge, psychological status, well-being, motivation).
Results: Mean age of patients was 12.4 ± 3.7, diab. duration 4.5 ± 3.3 ys and HbA_1c was 8.2 ± 1.5%, with only 19% (31/161 patients) having levels <7.0% (DCA 2000, mean normal 4.0–6.4%) (incidence of severe hypoglycaemia .004). In our hospital as part of the intervention 153/161 patients (95%) attended a structured treatment and teaching program (STTP) specially designed for children and adolescents (integrating insulin dose adjustment for normal eating, exercise, "real life training", psychological lessons, empowerment). Most frequent problems identified in the whole group were instability of blood glucose levels with a tendency towards hypoglycaemia in 79 patients (49%), in younger patients problems in insulin dose adjustment (in 50%) and self-monitoring (in 41%). 53/161 patients (33%) had motivational problems: Those patients were older (14.9 ± 2.2 vs 11.4 ± 3.5 ys, p<0.001), had a higher HbA_1c (8.9 ± 1.5 vs 7.6 ± 1.3%, p<0.001) and a longer diabetes duration (5.8 ± 2.9 vs 3.9 ± 3.2 ys, p<0.001). Using ANOVA at baseline there were no associations between theoretical knowledge and quality of diabetes control (p=0.089) or the incidence of hypoglycaemia (p=0.735), but there was an association between age and motivational problems (R-square=0.139, β=0.373, p=0.011). Following the STTP with "real life training" and psychological intervention a stabilisation of blood glucose levels (as indicated by lower blood glucose excursions during a period of 6 days) could be reached in 71/79 patients (90%). In older children and adolescents the problems in self-monitoring resulted mostly from motivational problems, in younger children a high percentage performed incorrect measurements: Systematic variations of± 15% of the true values were identified in 51 patients (32%).
Conclusion: In the pediatric age-group high percentages of patients have poor metabolic control. Reasons for this dramatic phenomenon are various and age-dependent: These are instability due to exercise and variations in eating in all age-groups, problems in insulin dose adjustment and self-monitoring more frequently in younger children and motivational problems mainly in adolescents. In a high percentage of patients these problems can be overcome by extensive examination with systematic strategies to identify problems followed by a specially designed STTP integrating exercises in insulin dose adjustment, self-monitoring, "real life training", psychological intervention and empowerment.

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Screening for eating disorders in young people with type 1 diabetes: comparative analysis with the general population in Argentina
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Background and Aims: To determine the prevalence of eating disorders (ED's) at a preclinical stage in adolescents with type 1 diabetes mellitus compared with that in their non-diabetic peers as determined through the study in the general population (GP) by the Argentine Pediatric Society (APS). To examine the relationships between disordered eating and insulin misuse with weight, glycemic control and socio-environmental factors
Materials and Methods: In a cross sectional study conducted in 13 pediatric centers specialized in diabetes all over the country, 270 randomly selected patients with type 1 diabetes aged 10 to 19 years (M= 112, F= 158) who had diabetes for more than one year, were assessed between December 2001 and August 2003. EDEQ4 (Eating Disorders Examination Questionnaire), modified with 40 questions and 5 scales was used for screening for ED's and EDE12 (Eating Disorder Examination) for diagnosis. The diagnostic criteria were taken from the DSM-IV. Socio-environmental factors were assessed through a query, glycemic control evaluated as the mean of the last 3 values of HbA_1c, height and weight were determined and BMI calculated, pubertal stage was described through Tanner stages. The results were compared with 1971 non-diabetic peers evaluated with the same tools in the study conducted by the ASP. *Chi*2, T test, Fisher test and Anova were used for statistical analysis. A p value <0.05 was considered significant. The statistical analysis was done using EpiInfo V. 6.04.
Results: 26.5% (n=72) of the 270 patients screened positive for EDEQ4 (i.e suspicious of ED's) vs. 19.2% (n=380) in the GP (p 0.001). More females than males (80% vs 20%) resulted suspicious of ED's. When compared to the GP the proportion of males who tested positive for EDEQ4 was higher among diabetics (20% vs. 12%, p 0.001). Regarding the distribution by age, the group aged 11y showed the higher difference in prevalence with the GP (37% vs.15%, p 0.01). There was a peak at 19 years in both populations, but higher for diabetics (42% vs 32%). There was a higher proportion of EDEQ-4 positives among pubertal than pre-pubertal females (p 0.006), patients with previous feeding problems (p 0.001) and those with a BMI over the 90th centile (p=0.03). Insulin misuse was present in 15% (n=11) of the EDEQ-4 positive patients. According to the scales in EDEQ-4, patients with diabetes showed more feeding preoccupation than those in the GP (p 0.00). The prevalence of overweight was 18.8% (n=51) in patients vs. 26.3% (n=518) in the GP (p NS). The EDE12 questionnaire was administered to 78% of the patients suspicious of ED's (n=56) and the diagnosed was confirmed in 53 (95%). All of them were NOS eating disorders according to the DSMIV. Of these, 48 (91%) fulfilled the criteria for binge eating disorder (BED). None met criteria for AN or BN.
Conclusion: We found a higher index of suspicion of ED's and of subthreshold disorders among the diabetic patients than in the GP and higher than reported by the literature. The treating team should be alert especilly with pubertad female, patients with a history of feeding problems and those with very high BMI.
Diabetes in Childhood Study Group (SAD): N. Buschenbaum, M. Ferraro, O. Ramos, M. Araujo, G. Krochik, B. Ozuna, S. Lopez, H. Raizman, L. Trifone, G. Freijo, E. Scaiola, R. Varela, S. Gonzalez, M. Pianessi, A. Cayssials, D. Setton, M. Honfi.

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Insulin aspart compared to regular insulin and insulin lispro in basal bolus therapy with NPH to treat pediatric patients with type 1 diabetes mellitus
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Background and Aims: A rapid-acting insulin analog such as insulin aspart (IAsp, NovoRapid®) can be administered immediately before meals and can provide an advantage over regular human insulin in children with type 1 diabetes who may have variable and unpredictable eating patterns.
Material and Methods: Efficacy and safety of basal/bolus therapy with IAsp +NPH were compared to regular human insulin (regular)+NPH or insulin lispro+NPH in this 24-week, randomized, open-label study in 378 children (6–18 yrs, HbA_{1c} ≤12% and type 1 diabetes ≥one yr). IAsp and lispro were administered immediately before meals; regular insulin, 20–30 min before meals; and NPH, before breakfast and before dinner or bedtime.
Results: Subjects had mean age (±SD): 11.6 ± 2.9 yrs, and BMI: 21.2 ± 4.7 kg/m^2. HbA_{1c} values (mean %SD) were 8.3 ± 1.2, 8.3 ± 1.3, 8.4 ± 1.2 at baseline, and 8.4 ± 1.4, 8.4 ± 1.4, and 8.2 ± 1.2 at the end of the study for the IAsp, regular, and lispro groups, respectively. The change-from-baseline HbA_{1c} value for the IAsp group (0.1%± 1.0) was not significantly different from either the regular group (0.1%± 1.0; treatment difference 97.5% CI: [–0.51%, 0.12%]) or lispro group (–0.1%± 1.0; 97.5% CI: [–0.06%, 0.54%]). Fasting plasma glucose (FPG) values were 12.4 ± 5.5, 13.2 ± 5.4, and 13.6 ± 5.4 mmol/L at baseline, and 14.0 ± 5.6, 13.1 ± 5.7, and 12.9 ± 5.3 mmol/L at the end of the study for the IAsp, regular, and lispro groups, respectively. The end-of-study FPG value in the IAsp group was not significantly different from the regular group or lispro group (97.5% CI for treatment difference: [–1.19, 2.50 mmol/L] and [-0.69, 2.87 mmol/L] for regular and lispro groups, respectively). Rates of minor hypoglycemia (confirmed by BG <2.8 mmol/L) were 24.2, 28.7, and 24.3 episodes/year, for the IAsp, regular, and lispro groups, respectively. Major hypoglycemia occurred at similar rates of 0.2, 0.3, and 0.2 episodes/year, respectively. Average daily insulin doses (basal/bolus) were similar at the end of the study (IAsp/NPH: 0.45/0.70; regular/NPH: 0.45/0.71; lispro/NPH: 0.43/0.67 U/kg).
Conclusion: Insulin aspart use in basal-bolus therapy with NPH is as safe and effective as lispro+NPH or regular insulin+NPH in treating pediatric patients with type 1 diabetes mellitus.
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Insulin treatment in type 1 diabetes

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Continuous subcutaneous insulin therapy in type 1 diabetic patients: retrospective study of about 500 patient-years

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Background and Aims: Continuous subcutaneous insulin infusion (CSII) using portable pumps is considered as the gold standard for the treatment of patients with type 1 diabetes who do not succeed in achieving good metabolic control with daily multiple insulin injections. The aim of the present study was to analyze the clinical results of CSII-treated type 1 diabetic patients in real life.
Material and Methods: Medical files of 95 type 1 diabetic patients (29 men and 66 women) treated with CSII and followed in one single centre were analyzed retrospectively. Specific interest was devoted to: 1) main indications of pump therapy; 2) HbA_{1c} levels; 3) severe hypoglycaemic or ketoacidotic episodes; 4) catheter-related local infections; and 5) pregnancy outcomes.
Results: Indications of CSII were heterogeneous, including allergy to insulin (n = 1), ongoing (n = 4) or programmed pregnancies (n = 24), recurrent hypoglycaemic comas (n = 16) and poor glycaemic control assessed by HbA_{1c} > 8% (n = 50). Mean (±SD) age was 43 ± 13 years and mean diabetes duration was 23 ± 12 years. Mean CSII follow-up averaged 5.1 ± 4.6 years, with a large distribution (< 5 years: n = 61; 5–10 years: n = 19; > 10 years: n = 15). HbA_{1c} levels before CSII was 8.6 ± 1.3% whereas mean values along CSII therapy averaged 8.3 ± 1.1% (p < 0.001). Last observed HbA_{1c} level was almost similar to average value throughout the follow-up (8.4 ± 1.0%). In fact, very heterogeneous results were observed among CSII-treated patients with rather few patients reaching a sustained optimal metabolic control: 5 with HbA_{1c} ≤7%, 66 with HbA_{1c} between 7.1 and 8.5%, 24 with HbA_{1c} > 8.5%. Sex, diabetes duration or type of pump did not significantly influence HbA_{1c} level in the tested population. Most discriminant parameter for good metabolic control was pregnancy (average HbA_{1c} of 6.8 ± 1.4% during 24 pregnancies in 14 women). However, 3 babies had malformations (1 cardiac and 1 renal), despite good glycaemic control before and during pregnancy in two cases. Globally, 97 severe hypoglycaemic episodes (including comas) were noted in 30 patients (incidence of 0.2 episodes/patient.year). Interestingly, patients in whom pump was initiated because of recurrent hypoglycaemic episodes with conventional therapy showed a reduction of the risk of severe hypoglycaemia while treated with CSII. Twenty-eight ketoacidotic episodes in 19 patients required hospitalisation (incidence of 0.05 ketoacidosis/patient.year), among which 11 could be attributed to an obvious technical problem. Twenty subcutaneous abscesses requiring a medical (oral antibiotics) or surgical (n = 1) treatment were observed in 13 patients.
Conclusion: CSII does not guarantee good glycaemic control in type 1 diabetic patients who were selected because of difficulties in obtaining adequate metabolic control with multiple insulin injection therapy. Follow-up in real life is less intensive (except in pregnant women) than that used in clinical trials, which may partly explain why HbA_{1c} targets were not reached in most patients. Initial and continuous education by a highly specialised team is a corner stone of the success, especially for reducing the number of acute complications and for reaching near normal HbA_{1c} levels.

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A randomized crossover study to compare continuous subcutaneous insulin infusion (CSII) with multiple daily injection (MDI) in type 1 diabetic patients previously treated with CSII

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Background and Aims: The aim of the study was to compare continuous subcutaneous insulin infusion (CSII) with lispro with multiple daily injection (MDI) therapy of bolus insulin lispro and basal insulin glargine.
Materials and Methods: 10 c-peptide negative type 1 diabetic patients (mean age 41 ± 8 years, duration of the disease 19.5 ± 10 years, HbA_1c 7.7 ± 0.7.%), previously on CSII therapy for at least 6 months, were studied during a randomized, crossover study. After one month run-in period, patients were randomized to receive either glargine or CSII therapy for a 4-month period and then crossed over to the other treatment. 48 hours continuous glucose monitoring was performed at the end of each study period with a microdialysis glucose sensor (Glucoday ®, A. Menarini Diagnostics, Firenze, Italy).
Results: Mean HbA_1c decreased to a similar extent after CSII and MDI therapy (7.2 ± 0.2 vs 7.2 ± 0.2, p=n.s). Continuous glucose monitoring profiles during 48 hour time period showed a significant lower glucose exposure during CSII treatment (mean glucose 147 ± 12 vs 189 ± 14 mg/dl, p<0.03, AUC >180 mg/dl 9603 ± 3941 vs 26445 ± 9390 mg/dl *3 min, p<0.02, glucose >65 mg/dl and < 180 mg/dl 1582 ± 212 vs 769 ± 158 min., p<0.02, CSII vs MDI respectively). Hypoglycaemic reactions exposure (AUC < 65 mg/dl) were similar in both treatment groups (1.88 ± 1.4 vs 2.63 ± 1.88 mg/dl * 3 min). During night-time (22.00 pm - 7 a.m.) CSII treated subjects spent significantly more time in the glucose range >65 mg/dl and <180 mg/dl than MDI treated patients (298 ± 63 vs 194 ± 51 min, p<0.02).
Conclusion: Continuous glucose monitoring data showed that CSII with insulin lispro provides lower nocturnal variability and better glycaemic control than MDI therapy with lispro and basal glargine without increasing the risk of hypoglycaemia. Both short- and long-acting insulin analogues allow acceptable daily plasma glucose control; yet, CSII-programmed s.c. insulin administration remains the reference standard for the treatment of type 1 diabetic patients

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Mechanisms and treatment of the "afternoon phenomenon" in patients with type 1 diabetes mellitus using glargine as basal insulin

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Background and Aims: To establish the mechanisms and treatment for the "afternoon phenomenon" (APh), an increase in blood glucose (BG) >50 mg/dl from 2-h post-lunch to before dinner which occurs in about one third of patients with tType 1 diabetes mellitus (T1DM) despite optimized use of rapid-acting insulin analog (RAIA) at meals (2-h post-lunch BG <150 mg/dl), and optimized use of glargine once daily at dinnertime (fasting BG<110 mg/dl).
Materials and Methods: Sixty T1DM patients (C-peptide <0.2 nmol/l) on such an insulin regimen, with confirmed APh, were randomized to a study on 2 test days at 1 week interval. On one day, patients had the usual RAIA dose with meals, on the other they fasted 24 hours skipping breakfast and lunch (as well as RAIA doses), but had same glargine dose on both occasions. The APh was 85 ± 14 mg/dl on fed, and 27 ± 3 mg/dl on fast day (p<0.05). Then the patients were randomized to 4 treatments for 6 months (15 patients in each of groups I-IV): I, continuation of RAIA at each meal and glargine once daily (dinner); II, as I, but human regular insulin (HRI) given at lunch in place of RAIA; III, glargine given twice/daily (50% of dose at breakfast, 50% at dinner); IV, as I, but 1-4 U of RAIA were injected 2.5–3.0-h after lunch in addition to usual pre-lunch RAIA dose.
Results: Neither the APh nor HbA_1c changed in group I. In group II, APh improved, but this was due to elevation of 2-h post-lunch BG with HRI, not decrease in pre-dinner BG, in fact HbA1c did not change. In group III, neither APh nor HbA_1c improved, rather nocturnal hypoglycaemia (BG<70 mg/dl between 12 pm-7 am) was more frequent (p<0.05 vs baseline and other groups). In group IV, APh improved from 79 ± 4 to 30 ± 2 mg/dl as well as HbA_1c from 7.24 ± 0.05 to 7.11 ± 0.05% (p<0.05).
Conclusions: the APh is not due to duration of action of glargine <24-h, but to insufficient insulin replacement with RAIA in the late part of lunch meal. Therefore APh treatment requires further optimization of insulin replacement at lunch with dual RAIA bolus.

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The impact of insulin glargine on lifestyle flexibility in patients with type 1 diabetes

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Background and Aims: This study was designed to investigate whether blood glucose remains adequately controlled with insulin glargine or NPH insulin treatment in patients with type 1 diabetes even if breakfast is missed in the morning. The effect on lipometabolism was also compared between the two regimens.
Materials and Methods: In this multicentre, open, parallel group study, patients with type 1 diabetes on intensified conventional insulin therapy with NPH insulin as the basal insulin were randomized to receive prandial insulin in combination with either bedtime insulin glargine (n=28) or NPH

insulin (n=32). Patients were initially titrated to target fasting blood glucose (FBG) levels ≥4.5 mmol/L and ≤7.2 mmol/L (≥80 mg/dL and ≤130 mg/dL) in the morning at 06:00–07:00 hours. Patients had no intake of any insulin or food between 22:00 hours and 12:00 hours the next day and blood glucose was determined at 01:00, 03:00 and 05:00 hours. If FBG levels were in the target range, blood glucose, non-esterified fatty acids (NEFA) and β-hydroxybutyrate (β-OHB) were determined hourly until 12:00 hours.

Results: At baseline, mean age (42.6 years), mean duration of diabetes (14.7 years), mean body mass index (25.6 kg/m^2) and mean levels of NEFA (0.5 mM), β-OHB (0.2 mM), HbA_{1c} (7.5%) and FBG (8.0 mmol/L [144.1 mg/dL]) were similar between the two treatment groups. At 22:00 hours, blood glucose levels were higher with insulin glargine versus NPH insulin (8.8 vs 7.2 mmol/L [158.2 vs 130.2 mg/dL]); however, from 22:00 to 12:00 hours the next day (the period of no insulin or food intake), blood glucose levels decreased with insulin glargine (-1.43 mmol/L [-25.8 mg/dL]) and increased with NPH insulin (+0.5 mmol/L [+9.1 mg/dL]; p=0.0284). At 22:00 hours, NEFA levels were similar in both treatment groups, but were significantly lower with insulin glargine than NPH insulin from 07:00 to 12:00 hours. The β-OHB levels were also similar at 22:00 hours in both groups, but were significantly lower with insulin glargine versus NPH insulin from 07:00 hours (0.19 vs 0.37 mM) to 12:00 hours (0.37 vs 0.72 mM); in addition, the β-OHB levels with NPH insulin were above the normal range (0.03–0.30 mM).

Conclusion: In summary, insulin glargine provides significantly better control of blood glucose and lipometabolism compared with NPH insulin in patients with type 1 diabetes on intensified conventional insulin therapy who miss breakfast and their insulin injection in the morning. Insulin glargine may thus permit flexibility in the insulin regimen.

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Introduction of insulin glargine to basal-bolus therapy improves metabolic control in patients with type 1 diabetes in everyday clinical practice

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Background and Aims: This study examined the effect of insulin glargine in basal-bolus therapy in type 1 diabetes patients with inadequate metabolic control in everyday practice.

Materials and Methods: In this 6-week, uncontrolled, observational study, 1942 patients with type 1 diabetes poorly controlled on their previous basal-bolus therapy (basal: 95.5% NPH insulin; 4.3% lente; 0.2% other; bolus: 43.5% human insulin; 29.7% insulin lispro; 23.3% insulin aspart; 3.5% other insulins) received insulin glargine (median time of application 20:00 hours) in combination with rapid-acting or regular human insulin. Dosing decisions were made at the physicians' discretion. The mean±standard deviation (SD) target fasting blood glucose (FBG) and HbA_{1c} levels set by the physicians were 6.3 ± 1.3 mmol/L and 6.6 ± 0.6%, respectively, after 6 weeks' treatment. Data relating to changes in HbA_{1c}, FBG, postprandial blood glucose (BG) and insulin dose are presented here.

Results: At baseline, mean±SD age was 42.5 ± 14.8 years, the mean±SD duration of previous basal-bolus treatment was 2.5 ± 3.4 years, mean±SD HbA_{1c} was 8.0 ± 1.3%, mean±SD FBG was 9.1 ± 2.5 mmol/L and mean±SD body mass index was 25.1 ± 4.0 kg/m^2. Patients achieved target FBG levels 6 weeks after the change in basal insulin (Table). At baseline, the mean±SD bolus insulin dose was 32.6 ± 17.6 IU (5.9 ± 4.4 IU/bread exchange unit); after 6 weeks, the mean±SD bolus insulin dose was 24.5 ± 14.6 IU (5.8 ± 4.1 IU/bread exchange unit). A total of 40 adverse drug reactions were reported in 17 patients, 20 of which were hypoglycaemic events.

	FBG (mmol/L)	HbA_{1c} (%)	2-hour postprandial BG (mmol/L)	Insulin glargine dose (IU)
Baseline	9.1 ± 2.5	8.0 ± 1.3	9.4 ± 2.6	20.9 ± 9.5*
6 weeks	6.6 ± 1.6	7.2 ± 0.9	7.4 ± 1.6	23.4 ± 10.1

*Mean starting dose of insulin glargine

Conclusion: These results from everyday practice are consistent with data obtained in clinical trials and suggest that replacing the basal insulin in basal-bolus therapy with insulin glargine may contribute to achieving target glycaemic control in patients with type 1 diabetes.

This study was supported by sanofi-aventis.

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Glargine vs insulatard: efficacy in comparison with insulin aspart in a basal bolus regimen in type 1 diabetes – the Glargine and Aspart Study (GLASS)

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Background and Aims: Recent trials show that the long-acting insulin analogue Glargine is as effective at improving glycaemic control as traditional basal insulins such as NPH, whilst resulting in less nocturnal hypoglycaemia. Previous studies used either regular soluble insulin or lispro as the meal-time insulin. We assessed the efficacy of insulin Glargine compared with NPH insulin when combined with the insulin analogue aspart in a 36 week cross-over trial.

Materials and Methods: GLASS is a randomised single centre open-label two-period crossover study comparing the effects on HbA_1c of the basal insulins Glargine and Insulatard combined with pre-prandial insulin Aspart. Inclusion criteria were T1DM subjects aged 18–75y with baseline HbA_1c 6–11% on insulin for at least 6 months. After four-week run-in, subjects were randomized to sixteen weeks' treatment with once-daily insulin Glargine or twice daily Insulatard, before crossing over to the other basal insulin for sixteen weeks. Insulin Aspart was continued as mealtime insulin for all patients throughout the study. Standardized local algorithm was used for both basal insulin groups and all underwent identical titration and visit schedules. The primary outcome measure was HbA_1c and secondary endpoints were fasting plasma glucose, weight change, incidence of hypoglycaemia and effects on lipid profile.

Results: 60 almost all White European patients (33 male, mean age±SD 42.7 ± 12.5y, diabetes duration 17.9 ± 12y) with T1DM were recruited. 53 completed the study. Baseline HbA_1c was 8.53%. After 16 weeks' treatment with Glargine HbA_1c was 8.05% and with Insulatard was 8.26% (mean difference 0.19%, 95% CI -0.36, -0.01). There was a significant difference between treatments (p=0.04).There was a highly significant difference between the insulins for change in fasting plasma glucose (p=0.002), with mean fasting glucose level 7.8 mmol/L after Glargine and 11.4 mmol/L after Insulatard. Mean (±SEM) basal insulin dose was 37 ± 2 IU/day of Glargine and 40 ± 3 IU/L/day of Insulatard (p=NS). After 16 weeks, Glargine dose was 41 ± 4 IU/L and Insulatard dose 36.7 ± 3 IU/L (p=0.1). There were no differences in minor hypoglycaemia (p=0.63), weight (p=0.45) or cholesterol (p=0.18) between groups. There was only one major hypoglycaemic event in each arm. Patient satisfaction was greater with Glargine and three patients dropped out of the study, as they did not wish to go back to Insulatard.

Variable	Glargine	Insulatard	Difference (Glargine-Insulatard)	95% Confidence Interval	p-value
HbA_1c (%)	8.07	8.26	–0.19	(–0.36, –0.01)	0.04
Fasting glucose (mmol/L)	8.42	11.42	–3.00	(–4.80, –1.20)	<.01
Incidence of hypos (%)	80.7%[1]	77.2%[2]	1.21*	(0.56, 2.64)*	0.63
Weight (kg)	81.68	81.92	–0.24	(–0.87, 0.39)	0.45
Cholesterol (mmol/L)	4.74	4.84	–0.10	(–0.25, 0.05)	0.18
Triglyceride (mmol/L) (Geometric means)	0.82	0.80	1.02*	(0.93, 1.12)*	0.63

*Ratio
[1] 2 subjects with no data, percentage calculated using total in group (57)
[2] 3 subjects with no data, percentage calculated using total in group (57)

Conclusions: Our study shows a small but significant improvement in glycaemic control with insulin Glargine without adverse effects on hypoglycaemia rate, weight, lipids or patient satisfaction. We would suggest that Glargine combined with insulin aspart is a satisfactory basal bolus regimen in subjects with type 1 diabetes.

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Reduction in insulin dose and body weight with pre- and post-meal insulin glulisine versus regular human insulin in patients with type 1 diabetes

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Background and Aims: People with diabetes may inject prandial insulin post-meal rather than pre-meal, but few studies have evaluated this practice. An open, multinational, randomized, controlled, parallel, 12-week study compared pre- and post-meal insulin glulisine (GLU) with regular human insulin (RHI).
Materials and Methods: Type 1 diabetes (T1DM) patients received once-daily insulin glargine plus: GLU 0–15 min pre-meal; GLU immediately at meal completion or 20 min after the start of the meal, whichever came first; or RHI 30–45 min pre-meal. Insulin doses were titrated to blood glucose (BG) targets while avoiding hypoglycaemia. This sub-analysis reports insulin dose and body weight data.
Results: Mean baseline characteristics were similar between the treatment groups (age 40.3 ± 11.7 years; body mass index 27.1 ± 4.7 kg/m^2; HbA_{1c} levels 7.7 ± 0.91% for GLU, 7.6 ± 0.92% for RHI). Baseline to endpoint changes in HbA_{1c} were similar for post-meal GLU and RHI (-0.11 vs -0.13%); the greatest reduction was with pre-meal GLU (-0.26%). Severe hypoglycaemic episodes were comparable for pre- and post-meal GLU vs RHI (8.4% and 8.4 vs 10.1%). A statistically significantly greater proportion of patients decreased total daily basal plus prandial insulin dose from baseline with both pre- and post-meal GLU vs RHI (46.1% and 46.6 vs 32.7%; p=0.0014 and p=0.0008, respectively). This was driven by changes in prandial insulin dose (Table). Body weight increased by +0.3 kg with pre-meal RHI and pre-meal GLU; however, there was a significant between-treatment reduction for post-meal GLU (-0.3 kg; p=0.03). Overall, fewer patients increased body weight with pre- and post-meal GLU versus RHI (50.7% and 44.8 vs 54.5%); this was statistically significant with post-meal GLU (p=0.0223). Furthermore, of these patients, fewer increased body weight >1 kg with pre- and post-meal GLU versus RHI (32.2% and 26.0 vs 37.6%); this was statistically significant with post-meal GLU (p=0.0035).
Conclusions: We conclude that in T1DM patients, glycaemic control with pre- or post-meal GLU is as effective as RHI, with less weight gain and fewer patients requiring increases in total insulin dose.

Reduction in daily prandial insulin dose (U)	Pre-meal insulin glulisine (% of patients; n=280)	Post-meal insulin glulisine (% of patients; n=283)	RHI (% of patients; n=269)
Any dose reduction (<0)	49.8*	47.0**	36.4
<-1	42.7*	38.9*	26.8
<-2	35.6*	32.5*	20.1
<-3	29.5*	27.2*	14.1
≤-4	26.0*	23.7*	12.6

* p <0.01 vs RHI; ** p <0.05 vs RHI; the dose reduction trends shown here were also similar for dose increases

This study was supported by sanofi-aventis

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Thrice-daily injections of biphasic insulin aspart 30 (NovoMix® 70/30) improves glycaemic control in patients with type 1 diabetes

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Background and Aims: Biphasic insulin aspart (BIAsp) 30 is compounded of 30% free and 70% protamine-bound insulin aspart. In addition to mealtime insulin it is anticipated that multiple mealtime injections of BIAsp would provide stable basal insulin levels, due to the fact that absorption from several smaller insulin subcutaneous depots may counterbalance each other in terms of variability. The primary objective of our study was to compare the effect of multiple mealtime injections of BIAsp30 to traditional basal-bolus human insulin administration (BBHI) on HbA_{1C} in type 1 diabetic patients.
Materials and Methods: Twenty-three type 1 patients participated, age 45(21-63) years (median and range), diabetes duration of 20 (2-45) years. Patients were randomly assigned to either BIAsp30 thrice-daily at mealtime supplemented with bedtime NPH if necessary or BBHI for 12 weeks, and then switched to the alternative regimen for another 12 weeks. At the end of each treatment, the patients attended two 24 h profile days one week apart for insulin pharmacokinetic and pharmacodynamic assessments. HbA_{1C} was measured at baseline and the end of each period. The insulin dosages were only kept constantly from the day before the first profile day to the end of each period.
Results: The treatment with BIAsp30 resulted in a greater reduction in HbA_{1C} (baseline / BBHI / BIAsp30 (%, median and range): 9.0 (8.1–12.3) / 8.6 (7.4–11.4) / 8.3 (6.7–9.8), *P<0.05*). The improvement in nighttime glycemic control was observed only with BIAsp30. The mean total daily insulin dose was not significantly different before and during the study and between the two trial regimens (baseline / BBHI / BIAsp30 (IU/24 h, geometric mean and range): 49.7 (24–106) / 50.4 (24–108) / 50.3(24–106), NS). However, the proportion of intermediate-acting insulin was increased from 38% (BBHI) to 75% (BIAsp30). Furthermore, 11 patients took bedtime NPH in addition to their thrice daily BIAsp30. Day-to-day variations in pharmacodynamics and pharmacokinetics and the frequency of hypoglycemia were not increased during treatment with BIAsp30.
Conclusion: Thrice daily injections of BIAsp30 can significantly improve long-term glycemic control without increasing the risk of hypoglycemia. Despite a higher proportion of intermediate-acting insulin, thrice daily injections of BIAsp30 did not increase the day-to-day variations in insulin pharmacokinetics and pharmacodynamics.
Support: Novo Nordisk A/S

PS 81

Economic implication of diabetes treatment

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Cost-effectiveness of atorvastatin (10mg) in patients with type 2 diabetes without coronary heart disease: CARDS trial analysis and extrapolation

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Background and Aims: To investigate the cost-effectiveness of lipid-lowering therapy with atorvastatin 10 mg in the primary prevention of cardiovascular disease (CVD) in patients with type 2 diabetes, using patient level data from the Collaborative Atorvastatin Diabetes Study (CARDS)
Materials and Methods: An incremental cost-effectiveness analysis was undertaken on the 2838 CARDS participants from the UK and Ireland with type 2 diabetes who were at risk of CVD. An incremental cost per cardiovascular event free year, based on the pre-defined primary and secondary trial outcomes for the within trial intent-to-treat population (ITT), a cost per life-year-gained and a cost-per-QALY were calculated. The primary trial endpoints were acute coronary heart disease (CHD) death; non-fatal MI including silent MI; hospitalised unstable angina; resuscitated cardiac arrest; coronary revascularisation; and stroke. A relative risk reduction (RRR) of 37% occurred in the atorvastatin arm. The secondary endpoints were total mortality and any cardiovascular endpoint, (RRR of 27% and 32% respectively occurred in the atorvastatin arm). An incremental cost-effectiveness analysis was undertaken for the within trial ITT population - median follow-up of 3.9 years. Costs were calculated at the individual patient level using unit costs from various UK published sources using 2003/04 prices. Extrapolation beyond the trial period to estimate a cost-per-life-year gained and cost-per-QALY was also undertaken using life expectancy estimates obtained from the general UK population that matched the placebo population in CARDS.
Results: Treatment with atorvastatin 10 mg raised net treatment costs by £521 per patient over the 3.9 years of study period (discounting at 3.5% per annum). The cost per event averted over the trial period, using the definition of primary endpoints averted, was £16,196. The incremental cost per event free year, based on fatal CHD and non-fatal MI events, was £7,608, while the incremental cost per event free year based on any cardiovascular endpoint was £4,896, and £4,119 when the events were defined as all study endpoints (any CVD event and any death) over the study period (discounting at 3.5% per annum). Extrapolation beyond the trial period calculated a gain in life years of 0.31 in the atorvastatin arm. This resulted in an incremental cost-effectiveness ratio of £7,540 per life year gained over lifetime (costs and effects discounted at 3.5%), and £4,030 per life year gained if effects are not discounted. Finally an incremental cost per QALY was calculated to be £7,318 (costs and effects discounted at 3.5%) and £3,884 per QALY if effects were not discounted.
Conclusion: Administering atorvastatin 10 mg for the primary prevention of CVD in patients with Type 2 diabetes, proven by CARDS to be safe and efficacious, is shown to be cost-effective falling well within the current threshold for cost-effectiveness set by the UK National Institute of Clinical Excellence (NICE).
Support: Pfizer Inc.

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The health economic value of Aspirin in the primary prevention of cardiovascular disease in diabetic patients in four European countries

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Background and Aims: Low-dose aspirin is standard care in patients with a history of cardiovascular disease (CVD). In primary prevention it is more controversial although recent meta-analyses and US and European guidelines support its use in persons at increased CVD risk, for example persons with diabetes. This study aims to evaluate the total costs and cost-effectiveness of low-dose aspirin in diabetic patients in the UK, Germany, Italy and Spain.
Materials and Methods: A simulation model was developed to compare the total cost of low-dose aspirin with placebo in the primary prevention of CVD over 10 years. The model was applied to patients with and without diabetes. Ten year cumulative risk of CVD in the studied patients groups was provided by the PROCAM-investigators (13.6% for the average diabetic patient vs. 5.4% for the average non diabetic patient). Risk reduction with low-dose aspirin was based on two recent meta-analyses. Direct costs from the perspective of the health care payer, i. e. excluding patient co-payment, were used (base year 2003). Outcomes are expressed as difference in 10-year total costs (€) and gains in quality-adjusted life years (QALY). Utility data for calculating the QALYs were obtained from published sources. Country specific discounting of future costs and QALYS were applied.
Results: As shown in the following table, administering low-dose aspirin is cost saving and leads to gains in QALYs in both populations studied in all countries, except Italy where for the non diabetic patients there is a very small incremental cost. The incremental cost-effectiveness ratio in Italy is only €193/QALY gained. The savings are the largest in diabetic patients. Sensitivity analyses proved robustness of the results. Cost savings in diabetic patients are statistically significant from year two on. Monte Carlo analysis showed cost savings in more than 95% of cases in the diabetic patients.

Difference in total costs and gains in quality-adjusted life years over 10 years

		Non Diabetic patients			Diabetic patients		
Country	Strategy	Total per patient (€)	Difference in total costs (€)	QALY gained	Total costs per patient (€)	Difference in total costs (€)	QALY gained
UK	No Aspirin	531			1,311		
	Aspirin	477	-55	0.020	1,116	-195	0.040
Germany	No Aspirin	634			1,566		
	Aspirin	576	-58	0.030	1,295	-271	0.040
Italy	No Aspirin	1,397			3,431		
	Aspirin	1,401	5	0.020	3,029	-402	0.040
Spain	No Aspirin	2,228			5,579		
	Aspirin	2,016	-211	0.030	4,810	-768	0.040

Conclusion: From the healthcare payer perspective, administering low-dose aspirin to patients with diabetes is cost-saving. Both on clinical and economic grounds, primary prevention with low-dose aspirin is recommendable in diabetic patients.
This study was supported by Bayer Healthcare AG

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Improving life expectancy and decreasing incidence of diabetes related complications: a modeling study of HbA_{1C} targets

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Background and Aims: To project the long-term clinical and cost outcomes that accompany predefined improvements in glycemic control in patients with type 2 diabetes.
Methods: A peer-reviewed, validated, non-product-specific Markov model of type 2 diabetes was used to project the long-term clinical and cost outcomes associated with four HbA_1c reduction scenarios (versus no reduction): 1) decreasing mean HbA_1c from 12.0% to 9.5%; 2) from 9.5% to 8.0%; 3) from 8.0% to 7.0%; and 4) from 7.0% to 6.5%. A typical US type 2 diabetes cohort based on NHANES data was simulated over a lifetime horizon (35 years). Diabetes-related complication costs (2003 USD) were accounted based on published data. Discount rates (3% *per annum*) were applied to clinical benefits and costs. Sensitivity analyses were performed.
Results: Stepwise reductions in HbA_1c as an independent variable correlated with delayed time to diabetes-related complications and reduced cumulative incidence of complications, including cardiovascular, renal and neurologic comorbidities. Related costs also decreased. Reductions in both poorly- (12.0% to 9.5%) and well-controlled (7.0% to 6.5%) patients produced incremental gains in undiscounted life expectancy (LE) (1.94 and 0.32 years, respectively). A similar pattern of improvements was observed in quality-adjusted life expectancy (QALE). Benefits from sequential reduction scenarios, when aggregated, exhibited the most dramatic effect. Sensi-

tivity analysis showed that these findings were robust under variations in time horizon and discount rates.

	Difference (HbA1c reduction – no reduction)		
	Undiscounted LE (years)	QALE (QALYs)	Lifetime cost of complications ($)
Scenario 1 (12.0–9.5%)	1.94 (0.28)	1.01 (0.13)	–14,487 (3,849)
Scenario 2 (9.5–8.0%)	1.06 (0.31)	0.56 (0.14)	–7,120 (3,546)
Scenario 3 (8.0–7.0%)	0.70 (0.33)	0.38 (0.15)	–3,831 (3,382)
Scenario 4 (7.0–6.5%)	0.32 (0.34)	0.17 (0.15)	–1,576 (3,060)

Values shown are means (standard deviation).

Conclusion: In this modeling study, improved glycemic control was associated with reductions in complication rates and costs, as well as increased LE and QALE in patients with type 2 diabetes. These data illustrate the long-term importance of reaching normal glycaemic control and support intensified HbA1c control as a cornerstone of effective long-term type 2 diabetes management.

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Comparing the incidence and lifetime costs for complications in male and female patients with type 2 diabetes in a managed care organization

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Background and aim: The objective of this study was to simulate the lifetime incidence and costs for complications in male and female type 2 diabetes patients of varying age within a large managed care organization (MCO).

Methods and materials: Baseline cohort characteristics from 14,587 patients with type 2 diabetes were retrieved from the MCO database: 7,672 female and 6,915 male. The gender groups were sub-divided in to groups of 18–45, 46–55, 56–64 and 64+ years. Simulations to compare gender-specific outcomes over patient lifetimes for each of the four age groups were performed using a peer-reviewed and validated computer simulation model that combines 15 different Markov sub-models to simulate the progression of diabetes and its complications. Transition probabilities were derived from the best available and most complete published sources, including UKPDS, USRDS. The costs of complications were taken imputed from published US sources. The analysis was performed from a third party payer perspective using recommended discount rates for the US setting (3.0% *per annum* on costs and outcomes).

Results: At baseline, mean ages were statistically similar between females and males in the 4 age groups. Females had more favorable mean HbA_1c in 3 of the 4 age groups and HDL in all age groups than males. Females also had more adverse BMI and LDL in all age groups.

Long-term projections showed that females had longer life expectancy than males in all four age groups. Remaining lifetime costs for the youngest three age groups were lower for females than males ($16,090, $7,576 and $1,312, respectively), and were comparable in the oldest age group.

Mean (standard deviation) values (female-male)	Age group (years) 18–45	46–55	56–64	64+
Difference	+1.22	+0.32	+0.54	+0.49
Life Expectancy (years)	(0.27)	(0.24)	(0.22)	(0.18)
Difference	-$16,090	-$7,576	-$1,312	-$103
Total costs	(4,240)	(4,253)	(3,960)	(3,391)

Conclusions: Gender is an important predictor of future life expectancy and costs of care. In this MCO, adult females with type 2 diabetes had longer life expectancy and lower remaining lifetime costs than males due, in part, to generally lower risks of future complications at baseline. Additional research is needed to understand reasons for gender disparities in risks of future complications of diabetes.

This study was sponsored with an unrestricted grant from Novo Nordisk Inc

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Direct costs of treatment for patients with type 1 or type 2 diabetes with or without complications in the USA

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Background and Aims: The increasing worldwide prevalence of diabetes and its complications is resulting in increasing disability, reduced life expectancy, and enormous costs in virtually every society. In the present study we sought to better understand the escalating cost of treating diabetes and its short- and long-term complications in the United States.

Materials and Methods: The direct healthcare costs of managing and treating type 1 or type 2 diabetes with or without complications were calculated using the PharMetrics Anonymous Patient-Centric Database (PDB). The PDB is the largest and most complete patient-centric data resource currently available, representing the medical and pharmacy records for a population of over 50 million patients from over 70 different, geographically diverse health plans. Patients were included if they were 18 years of age or over, had 2 or more claims with the International Classification of Diseases (ICD-9) code for diabetes, and 1 year of continuous enrollment. Costs assessed for patients without complications were outpatient treatment, hospitalizations, home care, patient education, glucose monitoring, lung function tests, liver enzyme tests, and drug therapy for hyperglycemia, hypertension, microalbuminuria, and dyslipidemia. Cost calculations began at diagnosis and continued for 1 year. For patients with complications, costs were subdivided for end-stage renal disease (ESRD, eg, dialysis), macrovascular disease (eg, myocardial infarction), hypo- or hyperglycemia, nervous system conditions, and ophthalmic disorders. Cost calculations began at the first episode and continued through available follow-up data. Costs were identified by ICD-9 and procedure codes (Correct Procedural Terminology [CPT], Health Common Procedures Coding System [HCPCS]) and annual costs calculated by follow-up. Charges served as a substitute for costs.

Results: Of all patients (N = 506,145) almost a quarter (24.7%) had a diabetes-related complication (mean age = 54 years; 51% male). Conditions associated with the highest costs were ESRD and macrovascular disease (range, US $46,585 ± 31,970 to US $135,275 ± 121,105/patient/year [PPPY]). Annual treatment costs for some chronic conditions (eg, heart failure) were higher than those for the first event (annual, US $36,864 ± 75,341 PPPY; first event, US $16,859 ± 36,790 PPPY). For patients without complications, highest costs were associated with hospitalizations (US $56,517 ± 78,246 PPPY), followed by medications and outpatient visits.

Conclusion: In the United States the greatest costs for diabetes care are related to complications such as ESRD and macrovascular disease. Hospitalizations, not outpatient treatment, accounted for the highest costs in the 75% of diabetes patients without known complications. Improvement of glycemic control remains one of the major therapeutic objectives for the prevention of life-threatening complications and reduction in hospital admissions related to diabetes. Therefore, a much more aggressive adoption of strict treatment goals and effective treat to target clinical decision making strategies must be implemented if we are to help reduce the billions of dollars currently spent on diabetes worldwide.

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Is intensive medical management in patients with type 2 diabetes and nephropathy cost effective?

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Background and Aims: Diabetes now accounts for 18% of patients starting dialysis in Scotland. Factors known to affect progression of diabetic nephropathy are well known and by targeting these factors it may be possible to delay dialysis. We set up a 2-year randomised controlled study to assess the effect of intensive management on the rate of decline of renal function in patients with type 2 diabetes and nephropathy.

Materials and Methods: Ninety patients were recruited and randomised to intensive medical management (n=47) or standard medical practice (n=43). Clinical targets were the same in both groups. An economic analysis was performed to determine if intensive medical management was cost effective.

Results: The intensive group (IG) had a mean of 19 clinic visits over 2 years, spent a total of 496 days in hospital and progressed at a rate of 0.14 ml/min/month. The control group (CG) had 8 visits, spent 842 days in hospital and progressed at 0.53 ml/min/month. Three patients in CG started haemodialysis compared to none in the IG. The additional 11 clinic visits in IG cost 2,572 Euro per patient and additional drug costs were 581 Euro per patient. However, the reduction in hospitalisation saved 3,323 Euro per patient. The dialysis costs in the CG were 74,434 Euro or 1,731 Euro per patient in the CG. The net savings achieved by intensive medical management during the 2 years of the study were 1,901 euros per patient.
Long term savings could also be made. Assuming the rates of progression remain unchanged the patient in the IG would start dialysis at 27 years compared to 7 years in the CG. A patient in the CG could have 20 more years on dialysis costing 661,642 Euro. Even if there were only a 10% chance of this happening, which would take into account the high mortality rate, both pre-dialysis and on dialysis, then the savings would be 66,164 Euro per patient. Delaying dialysis should result in improved quality of life. Diabetes has a utility value of 0.9, while hospital dialysis has a utility value of 0.53. A gain of 0.37 for 20 years would result in 7.4 quality adjusted life years (QALYs). Actual cost of intensive management was 1,576 Euro per annum and 31,530 Euro for 20 years resulting in a cost per QALY of 4,261 Euro.
Conclusion: Intensive medical management of patients with type 2 diabetes and nephropathy is both clinically and cost-effective.

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Cost-effectiveness analysis of medical intervention in type 2 diabetic subjects in a tertiary care centre in Bangladesh

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Background and Aims: The economic burden resulting from diabetes related complications consume a major portion of resources allocated for health-care services. Cost-effectiveness of various interventions on type 2 diabetes and its complications have relatively been well explored in developed countries, but these are almost absent in developing countries. The present study was undertaken to assess the cost-effectiveness of medical intervention in type 2 diabetic subjects.
Materials and Methods: Two hundred type 2 diabetic patients with at least 1 yr of follow-up, were purposively selected from a tertiary diabetes care hospital. Of them 100 were poorly managed (group-1) and 100 were properly managed (group-1I). A comparison was made between the groups. The degree & extent of complications, treatment outcome, clinical effectiveness and direct, indirect & incremental cost of complications were calculated. Cost included hospitalizations, visits, diagnostics & pharmacological therapies. Clinical information included the information on complications like retinopathy, cardiopathy, vasculopathy, & nephropathy. Analysis was done regarding annual medical costs and complications using appropriate statistical analysis.
Results: A total of 200 diabetic patients were considered for an average of 365 days and it amounted to 651 person-yrs of observation in total. The mean (±SD) age of the groups was 53.6 ± 8.8 yrs in group-1 & 55.0 ± 9.1 yrs in group-II. Systolic blood pressure were found as 152.5 ± 20.9 mmHg & 123.5 ± 11.9 mmHg, diastolic 97.7 ± 10.0 & 78.7 ± 9.3 mmHg, total cholesterol 195.5 ± 41.6 & 109.2 ± 34.5 mg/dl, HDL cholesterol 50.2 ± 20.3 & 39.1 ± 15.1 mg/dl, HbA_1c 7.1 ± 1.5% & 4.9 ± 1.9%, and Hb level 11.9 ± 1.3 & 13.9 ± 1.5 g/dl respectively. 19% diabetic patients were free of diabetic complications in group-1 & 36% in group-II. In group-1, 32% had one complication, 29% had two & 20% had more than two complications. On the other hand, in group-II, the corresponding values were 48%, 10% & 6% respectively. The most frequent complication was vasculopathy, which affected 32% patients in group-1 & only 26% in group-2, followed by cardiopathy 22% & 19%, retinopathy 16% & 12%, & nephropathy 11% & 7% respectively. The average annual cost of care was US$ 27616.32 (direct US$ 18593.12 & indirect US$ 9023.2) with an average US$ 138.08 per patient. 52% (US$ 14360.48) of costs were attributable to drugs for both groups of which US$ 10419.39 was for group-1. 28% (US$ 7732.56%) to hospitalizations of which US$ 4913.72 was for group-1. 11% (US$ 3037.79%) to diagnostics and US$ 1953.22 for group-1 and 9% (US$ 2485.46) to visits and US$ 1631.42 for group-1. The annual medical costs increased with the number of complications from US$ 1,320 to US$ 2,296 and to US$ 3,989 in group-1 with one, two and more than two complications which is increasing at a rapid speed and US$ 917 to US$ 1556 and to US$ 2372 in group-II respectively, increasing at a diminishing marginal rate. The regression equation showed that medical cost is significantly related to complications tested in both univariate ($P < 0.0001$) and multiple linear regression analyses ($R^2 = 0.21$; F-82.5, $P < 0.0001$).
Conclusion: Proper management with regular follow up substantially reduces the expenditure related to care of diabetes and its complications even in a developing country. Strategies aimed at preventing the onset of diabetes complications are likely to reduce medical costs in the long term, while improving patients' health.

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Payment for clinical audit standards improves diabetes care in United Kingdom

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Background and Aims: The prevalence of diabetes and the cost of providing services are likely to increase dramatically over the next ten years. One way forward is for primary care to provide more routine care for people with diabetes. There is now good wealth of evidence to show that early diagnosis and good management of the risk factors can reduce the risk of premature death and complications. The new General Medical Service contract rewards each of the primary care practices for the quality provided to improve clinical standards in Diabetes Care. The aim of the study was to assess the usefulness of the new payment scheme to improve Diabetes Care using the Alphabet Strategy audit tool in primary care.
Materials and Methods: Twelve General Practice surgeries that provided care for 3173 patients with diabetes in North Warwickshire, United Kingdom were selected. Initial assessment on the clinical standards from the new General Medical Service contract in Diabetes was carried for the pre-contract year 2004 using the Alphabet Strategy format. The data on the practices performance were collected from the Primary Care Trust database. The twelve General Practice surgeries were re-assessed again for the post- contract year in 2005 for the same quality indicators. Results were compared using the Chi-squared test and the paired Students t-test.
Results: There was a significant improvement in the clinical standards such as blood pressure, $HbA_{1c}{}^{<10}$, eye and foot care and the usage of ACE inhibitors or angiotensin receptor blockers and non-significant improvement in smoking advice, total cholestrol <5 mmol/L and HbA_{1c} <7. Results are summarised in the table shown below.
Conclusion: Financial incentives has a significant beneficial effect on several risk factors with the potential to improve Diabetes Care for patients and reduce morbidity and mortality due to diabetic complications. This contract therefore is expected to reduce cardiovascular disease, vision loss due to diabetic retinopathy and progression to end stage renal failure due to diabetic nephropathy.

Comparison of General Medical Service contract quality indicators in the Alphabet Strategy format

Degree of Diabetes care	Pre contract (range%)	Post Contract (range%)	% increase	p value
***A*dvise:** %DM who smoke and have been offered advice on smoking cessation	65.8 (25–100)	90.4 (77–100)	24.6	Not significant
***B*lood pressure:** %DM whose last BP is 145/85 or less	50.2 (15.5–89)	65.9 (46–92.7)	15.7	0.001
***C*holesterol:** %DM whose last Total Cholesterol is 5 or less	47.0 (36–58.2)	68.0 (56–91.6)	21.0	Not significant
***D*iabetes control:** %DM with HbA1c% of 7.4 or less	43.9 (27.2-70)	65.8 (57.6–88.5)	21.9	Not significant
***D*iabetes control:** %DM with HbA1c% of 10% or less	77.3 (49.5–95)	88.4 (81.5–96.8)	11.1	0.009
***E*ye:** %DM with record of retinal screening	60.0 (30.4–74)	74.4 (43.3–89.5)	14.4	0.004
***F*oot care:** %DM with a record of testing for peripheral pulses	56.1 (33.6–86)	72.7 (45.2-92.3)	16.6	0.0006
***G*uardian drug:** %DM with proteinuria or microalbuminuria who are treated with ACE-I or AII Blockers	61.4 (42.1-80)	78.5 (0–100)	17.1	0.0002

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Economic implication of insulin treatment

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Cost-effectiveness analysis of basal-bolus therapy of type 1 diabetes using insulin detemir+insulin aspart versus NPH+insulin aspart regimens

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Background and Aims: The results of a recent clinical trial showed that basal/bolus treatment of 408 sub-jects with type 1 diabetes with insulin detemir+insulin aspart (IDet/IAsp) signifi-cantly improved HbA1c (0.18%-points lower after 16 weeks) and body weight (-0.73 kg) compared to a regimen of neutral protamine hagedorm insulin+insulin aspart (NPH/IAsp). The aim of this modeling analysis was to estimate the long-term clinical and cost outcomes associated with IDet/IAsp and NPH/IAsp regimens in the German setting.
Materials and Methods: A validated, peer-reviewed computer simulation model (CORE Diabetes Model) was used to project the incidence of complications, life expectancy, quality-adjusted life expectancy, costs and cost-effectiveness over patient lifetimes. The model simulated progression of complications (cardiovascular disease, neuropathy, renal and eye dis-ease) based on a series of standard Markov sub-models running in parallel. Transi-tion probabilities and risk adjustments were derived from published clinical and epi-demiological studies. Baseline cohort characteristics and treatment effects were taken from the 16-week clinical study. Direct and indirect costs of diabetes complications and treatments were retrieved from published sources and accounted from a German Healthcare System perspective. A discount rate of 5% was applied to costs and clinical benefits.
Results: Long-term basal/bolus therapy with IDet/IAsp was projected to decrease the inci-dence of diabetes-related complications, and improve life expectancy (0.08 life years gained) and quality-adjusted life expectancy (0.09 quality-adjusted life years [QA-LYs] gained) compared to NPH/IAsp. Lower complication costs in the IDet/IAsp arm partially offset the increased costs of treatment. Mean total lifetime costs were €948 per patient higher with IDet/IAsp than with NPH/IAsp, leading to incremental cost-effectiveness ratios of €11,850 per life year gained and €10,533 per QALY gained.
Conclusion: Based on short-term clinical trial findings, IDet/IAsp was projected to reduce the in-cidence of long-term complications, improve life expectancy and quality-adjusted life expectancy, and can be considered to represent good value for money by German and international standards.
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Long-term cost-effectiveness analysis of patients with type 2 diabetes mellitus inadequately controlled on thiazolidinedione therapy and either biphasic insulin aspart 30 or sulfonylurea

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Background and Aims: To evaluate the lifetime (35 years) cost-effectiveness of a premixed insulin analog (BIAsp 30) plus thiazolidinedione (TZD) versus sulfonylurea (SU) plus TZD among poorly controlled type 2 patients in the US.
Methods and Materials: Data from a recent randomized clinical trial were used to calculate the long-term cost-effectiveness of two treatment regimens for type 2 diabetes mellitus. The trial was an open-label clinical study where type 2 diabetes insufficiently controlled on oral antidiabetic therapy (mean HbA1c: 9.5%) were allocated to receive treatment with BIAsp 30 (30% soluble insulin aspart, 70% insulin aspart crystallized with protamine)+TZD or SU+TZD over 18 weeks. Treatment with BIAsp 30+TZD exhibited significantly better HbA_1c reductions from baseline than SU+TZD (-1.2% versus -0.4%, $p<0.05$). A validated, peer-reviewed computer simulation model combining published data on the risk of long-term diabetic complications with quality-of-life utilities was used to simulate clinical and cost outcomes. The model employed standard Markov/Monte Carlo techniques to describe the incidence and progression of diabetes-related complications over patient lifetimes. Costs were accounted as complication expenses plus annual direct pharmacy costs using end-of-study dosing (US Medicare perspective). Clinical outcomes and costs were discounted at 3% per annum. Sensitivity analyses were performed.
Results: Improvements in glycemic control with BIAsp 30+TZD treatment resulted in reduced rates of vascular, renal, retinal, and neurologic complications compared to SU+TZD. Gains in discounted and quality-adjusted life expectancy were also reported with BIAsp 30+TZD (+0.39 and +0.32 years, respectively). The incremental cost-effectiveness ratio (ICER) was $25,400 (USD) per quality-adjusted life year (QALY) gained for BIAsp 30+TZD versus SU+TZD. Sensitivity analyses indicated that the findings were robust.
Conclusion: Due to improved glycemic control, BIAsp 30+TZD was projected to be cost-effective compared to SU+TZD in the treatment of patients with type 2 diabetes insufficiently controlled on a preexisting oral antidiabetic therapy. The ICER of $25,400 per QALY gained is within the cost-effectiveness threshold taken to represent good value for money in the US setting.
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Cost to reach target HbA_{1C} in poorly controlled patients with type 2 diabetes mellitus on either biphasic insulin aspart 30 and metformin or a maximized oral hypoglycaemic regimen

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Background and Aims: Prominent studies such as the United Kingdom Prospective Diabetes Study (UKPDS) reveal that reaching optimal HbA1C levels should be the primary goal of treatment for patients with type 2 diabetes. The direct pharmacy costs associated with two HbA1c-lowering strategies were evaluated when aiming to reach an HbA1C of ≤ 7.0% in type 2 subjects insufficiently controlled despite receiving oral hypoglycemic agents (OHA; 86.2% on dual therapy). Such patients are often placed on add-on or maximized OHA dosing as opposed to initiation of insulin. Thus, twice-daily biphasic insulin aspart 30 plus metformin (BIAsp 30+met) was compared with maximally tolerated dosing of an existing OHA regimen.
Methods and Materials: Efficacy data on these approaches were derived from a head-to-head, randomized clinical trial among insulin naïve, type 2 subjects previously uncontrolled on OHA therapy alone (mean baseline HbA_1c: 10.3%). The primary efficacy measure was the proportion of patients reaching the American Diabetes Association and International Diabetes Federation endorsed HbA_1c goal of ≤ 7.0% (i.e., the ratio of successfully treated patients). Direct pharmacy costs (2004 USD) were accounted using end-of-study mean daily medication doses of 44.8 units BIAsp 30 + 1380 mg metformin and, in the maximized OHA arm, 16 mg Glyburide, 5.25 mg Glimepiride, 7.3 mg Repaglinide, 1407 mg metformin, and 22.5 mg Pioglitazone, where applicable. Medication costs were based on published average wholesale prices (AWP) within the US. The cost per successfully treated patient, as an indicator of *cost efficacy* for each cohort, was obtained by comparing total pharmacy costs to success rates as a ratio. In the primary analysis, results were calculated over the trial period of 16-weeks, however, we also report extrapolated results based on annual pharmacy costs.
Results: Over the 16-week trial period, significantly more insulin-naïve, type 2 subjects previously uncontrolled with OHA therapy alone reached the target of $HbA_1c \leq 7.0\%$ with twice-daily BIAsp 30+met compared to maximized OHA therapy (45.0% vs. 26.2%; $p<0.02$). Total mean direct pharmacy costs per patient over the trial period were $639 and $487, respectively. The average costs per successfully treated patient were $1,419 (i.e., $639:45.0%) and $1,860 (i.e., $487:26.2%), respectively (Table 1). Annually, the average costs per successfully treated patient were $4,683 with BIAsp 30+met and $5,579 with optimized OHA therapy.
Conclusion: Treating previously uncontrolled type 2 diabetes patients with twice-daily BIAsp 30+met appears to be significantly more effective than seeking to optimize current OHA therapy. Our results indicate the average cost of bringing these patients within clinically recommended HbA1C levels is considerably less and a potentially better investment of healthcare dollars.

Table 1. Total Direct Pharmacy Costs and Success Rates to Goal

	BIAsp 30 + met	Optimized OHA
Percentage (%) of Patients Successfully reaching $HbA_1c \leq 7.0\%$	45.0%	26.2%
Total Direct Pharmacy Costs Per Patient (2004 USD)	$639	$487
Trial Period Cost Per Successfully Treated Patient	$1,419	$1,860

This study was sponsored with an unrestricted grant from Novo Nordisk Inc.

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Estimating the long term cost-effectiveness of biphasic insulin aspart plus metformin versus optimization of oral hypoglycemic agents in insulin-naïve patients with type 2 diabetes in a US cost setting

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Background and Aims: In a recent randomized clinical trial (RCT) comparing a combination of biphasic insulin aspart (BIAsp 30, 30% soluble and 70% protaminated insulin aspart, NovoMix® 30) plus metformin with oral hypoglycemic agents (OHAs) in insulin-naïve patients with type 2 diabetes, BIAsp 30 + metformin therapy was shown to significantly improve glycemic control compared to optimized OHAs over 16 weeks of treatment (difference in HbA_1c reduction: 0.85% (95%CI 1.16, 0.54), p<0.001). No major hypoglycemic events occurred during the trial and increases in BMI were comparable in both treatment groups. The objective of the present analysis was to project the long-term clinical and cost outcomes of treating patients with these interventions based on the findings of the RCT in a US cost setting.
Materials and Methods: Long-term projections were made using a peer-reviewed, published and validated model of diabetes. The simulated cohort was created using baseline characteristics of the RCT population (mean age 58.3 years; 21.2% male; mean HbA_1c 10.3%; duration of diabetes 9 years). The model was used to simulate the progression of the cohort over patients' lifetimes, taking into account the range of diabetes-related complications (eye, renal, cardiovascular and neurological disease) with effects of treatment applied as reported in the RCT. Lifetime costs in 2004 USD ($) were accounted as annual direct pharmacy costs and complication costs from a third party payer perspective (US Medicare). Discount rates of 3% per annum were applied to costs and clinical outcomes. Sensitivity analyses were performed.
Results: BIAsp 30 + metformin combination therapy was projected to improve life expectancy and quality-adjusted life expectancy compared to OHAs, and reduce the incidence of diabetes-related complications. Total lifetime direct costs were higher in the BIAsp 30 arm than with OHAs (due to greater treatment costs, but partially offset by reduced costs of complications), which led to incremental cost-effectiveness ratios (ICERs) of $ 8,049 per life year gained and $ 8,487 per quality-adjusted life year (QALY) gained for BIAsp 30 + metformin versus OHAs.
Conclusion: Treatment with BIAsp 30 was projected to reduce the incidence of diabetes-related complications, and improve life expectancy and quality-adjusted life expectancy compared to optimized OHA therapy in insulin-naïve patients. With a projected ICER of $ 8,487 per QALY gained, BIAsp 30 + metformin represents very good value for money by generally accepted US standards.

Results

	BIAsp 30 + Metformin	OHAs	Difference
Life expectancy (years)	12.41 (0.16)	11.94 (0.17)	0.47 (0.22)
Quality-adjusted life expectancy (QALYs)	8.46 (0.11)	8.20 (0.12)	0.44 (0.15)
Lifetime costs ($)	84,709 (2,310)	80,965 (2,449)	3,744 (3,200)

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Economics of basal insulin added to oral agents versus twice-daily premixed insulin as initial therapy for type 2 diabetes

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Background and Aims: This study models the effect of pharmacotherapy options on near-term (three year) societal costs for the treatment of type 2 diabetes mellitus (DM).
Materials and Methods: Data were derived from the medical literature, and a 24-week, multinational, multicenter, open, parallel group trial of 371 insulin-naive patients with inadequate glycemic control (FBG ≥120 mg/dL; HbA_{1c} range: 7.5–10.5%) who were prescribed an oral antidiabetic agent(s) (OADs), and subsequently randomized to: (i) once-daily insulin glargine (G), plus glimepiride and metformin; or, (ii) 30% regular/70% human NPH insulin (70/30) twice-daily. Stochastic decision-analytical modeling (10,000 iterations) was used to discern the economic impact (societal view) of treatment options on pharmacotherapy costs, hospital costs due to severe hypoglycemia (<36 mg/dL), effect of reduced HbA_{1c} on medical costs (exclusive of hospital costs associated with severe hypoglycemia), and productivity, over the first three years post-treatment. Modeling was conducted across age (30–80 years), U.S. gender balance for the prevalence of type 2 DM, and weight. Results are reported in 2004 U.S. dollars.
Results: Mean (age, gender balance, weight) findings with G+OADs include: (i) a significant increase in pharmacotherapy costs ($2,979.99/three patient-years; $p \leq 0.001$); (ii) a significant decrease in hospital costs due to severe hypoglycemia (-$1,732.50/three patient-years; $p \leq 0.001$); (iii) a significant decrease in medical service use due to reduced HbA_{1c} (-0.34%; $p=0.0003$) (-$994.50/three patient-years; $p \leq 0.001$); (iv) a significant decrease in productivity loss (-$273.00/three patient-yrs; $p \leq 0.001$); and (v) a net societal impact of -20.01/three patient-years (p=NS). Findings were robust via sensitivity analysis.
Conclusion: The near-term clinical benefits of using G+OADs (inclusive of: (i) a mean HbA_{1c} decrease from baseline (-1.64 vs. -1.31%; $p=0.0003$); (ii) a greater likelihood of reaching $HbA_{1c} \leq 7.0\%$ without nocturnal hypoglycemia (45.5 vs. 28.6%; $p=0.0013$); (iii) a decrease FBG (adjusted mean difference -17 mg/dL; $p<0.0001$); (iv) a greater likelihood of reaching target FBG ≤100 mg/dL (31.6 vs. 15.0%; $p=0.0001$); and (v) fewer confirmed hypoglycemic episodes (mean 4.07 vs. 9.87/patient-yr; $p<0.0001$)) were achieved without additional societal cost.
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A societal evaluation of the cost-utility of insulin detemir compared to insulin glargine, both in combination with insulin aspart in type 1 diabetes across five Western European countries

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Background and Aims: Intensive insulin treatment is associated with an increased risk of hypoglycemic events in type 1 diabetes. A recent 26-week randomized clinical trial demonstrated that basal/bolus treatment of 322 type 1 diabetic patients with insulin detemir+insulin aspart (IDet/IAsp), compared to insulin glargine+insulin aspart (IGlar/IAsp), led to a 72% lower risk of major hypoglycemic events (p<0.05). The aim of this modeling study was to project these short-term outcomes to evaluate long-term complication rates and associated costs with these regimens across five western European settings: Finland, Germany, The Netherlands, Austria and Spain.
Materials and Methods: A validated model was used to project long-term complications, improvements in quality-adjusted life expectancy, long-term direct and indirect costs, and incremental cost-effectiveness ratios (ICERs) for IDet/IAsp versus IGlar/IAsp. The model combined 15 Markov-based sub-models to simulate the ncidence and progression of diabetes-related complications (cardiovascular disease, neuropathy, renal and eye disease). Probabilities of complications were derived from the DCCT, Framingham and WESDR studies. Clinical inputs (baseline cohort characteristics and treatment effects) were taken from the 26-week international, open-label, parallel-group phase 3 trial in type 1 diabetic patients. Costs of treating complications were retrieved from published sources. Direct and indirect costs of diabetes complications and treatment with IDet/IAsp or IGlar/IAsp were projected over patient lifetimes from a societal perspective. Costs and outcomes were discounted at 5% per annum.

Results: The reduction in major hypoglycemic events associated with IDet/IAsp versus IGlar/IAsp was projected to lead to a lower cumulative incidence of diabetes-related complications. Quality-adjusted life expectancy was increased by 0.11 quality-adjusted life years (QALYs). Total lifetime costs were lower in the Netherlands, but slightly higher in the other four countries for IDet/IAsp versus IGlar/IAsp. As a result, IDet/IAsp was dominant to IGlar/IAsp in the Netherlands (life- and cost-saving). ICERs of €17,900 (Finland), €2,556 (Germany), €22,807 (Austria) and €475 (Spain) per QALY gained for IDet/IAsp versus IGlar/IAsp were projected in the other four countries.
Conclusion: The significant reduction in major hypoglycemic events associated with IDet/IAsp treatment was projected to lead to a decreased cumulative incidence of complications, reduced costs of complications, and improvements in quality-adjusted life expectancy in patients with type 1 diabetes. This modeling study indicated that IDet/IAsp treatment would be dominant to IGlar/IAsp in the Netherlands, and ICERs for the other four countries investigated would fall within the range considered to represent excellent value for money over patient lifetimes.
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Long-term evaluation of the cost-effectiveness of biphasic insulin aspart 30 versus insulin glargine in poorly controlled patients with type 2 diabetes receiving oral antidiabetic agents
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Background and Aims: Baseline population characteristics and end-of-study treatment effect data from a recent randomized control trial were used to simulate the long-term clinical and economic outcomes of two competing insulin regimens among type 2 diabetes patients failing to achieve glycemic control with oral antidiabetic agents alone (OADs).
Methods and Materials: Efficacy data on these therapies were derived from a multicenter, 28-week clinical trial (INITIATE) which demonstrated significant improvement in HbA1c levels (-0.43%, p<0.005) among insulin naïve type 2 patients randomized to twice-daily biphasic insulin aspart 30 (BIAsp 30) + metformin (met)±thiazolidinedione (TZD) compared to bedtime insulin glargine + met±TZD. A peer-reviewed, independently validated Markov/Monte-Carlo model combining published literature for risk of long-term diabetic complications with quality-of-life utilities was used to project the incremental cost-effectiveness ratio (ICER) as well as cumulative incidences of diabetes-related complications over a time horizon of 35 years. Cost-effectiveness was measured as cost per life years gained (LYG) and cost per quality adjusted life years gained (QALY). Cardiovascular, neurologic, renal, and retinal complication rates were assessed. Lifetime costs were calculated as the annual direct pharmacy costs for medications plus costs for complications (US Medicare perspective). Clinical outcomes and costs were discounted at 3% per annum. Sensitivity analyses were performed.
Results: Over the measured time horizon, improvements in glycemic efficacy corresponded with increases in LYG and QALY favouring BIAsp 30 versus glargine (0.20 ± 0.21 and 0.20 ± 0.15 years, respectively). Treatment with BIAsp 30 was also associated with reductions in the cumulative incidence of diabetes-related complications, notably in renal (16% decrease in end-stage renal disease) and retinal (9% decrease in severe vision loss) comorbidities. An ICER of $39,600 per QALY gained was deduced. Sensitivity analyses support the validity and reliability of the results.
Conclusions: Due to improved efficacy, BIAsp 30 was estimated to reduce lifetime complication incidences and be within cost-effectiveness thresholds when compared to insulin glargine in the treatment of patients with type 2 diabetes insufficiently controlled on OADs alone.

Table 1. Baseline Reductions in HbA1c and Projected Gains in Life Expectancy

	BIAsp 30 + met±TZD	Glargine + met±TZD
End-of-Study Mean Reductions in HbA1c from Baseline	–2.79%	–2.36%
Estimated LYG over a Lifetime Period	13.47 ± 0.17	13.27 ± 0.16
Estimated QALY gained over a Lifetime Period	9.39 ± 0.12	9.19 ± 0.11

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Health economic evaluation of long-term treatment with insulin aspart versus human insulin for type 1 diabetes in nine European countries
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Background and Aims: In a multinational open-label randomized clinical trial (RCT), intensive treatment with insulin aspart as the bolus component of a basal/bolus regimen was associated with improved glycemic control (0.12% lower HbA1c, P<0.02) compared to human insulin after 6 months in patients with type 1 diabetes. Insulin aspart was also associated with a non-significant decrease in the risk of major hypoglycemia events. The aim of this health economic study was to assess the cost-effectiveness of insulin aspart versus human insulin in patients with type 1 diabetes, based on the findings of this RCT, in nine European countries.
Materials and Methods: The CORE Diabetes Model, a peer-reviewed, validated computer simulation model, was used to project long-term clinical and cost outcomes of treatment. The model combined standard Markov sub-models to simulate the progression of disease and incidence diabetes-related complications (cardiovascular disease, neuropathy, renal and eye disease). Monte Carlo simulations were carried out over a patient lifetime horizon. Probabilities and HbA1c-dependent risk adjustments were derived from published sources. Treatment effects, dosing information and baseline cohort characteristics were based on the findings of the RCT. Only direct medical costs (treatment and complications) were considered. Discounting was performed according to country-specific guidelines. Extensive sensitivity analyses were performed.
Results: Insulin aspart was projected to be both life- and cost-saving versus human insulin in Austria, France, the Netherlands and Norway. In the Danish, Finnish, German, Spanish and Swedish, settings, incremental cost-effectiveness ratios (ICERs) for insulin aspart versus human insulin were below €30,000 per quality-adjusted life year (QALY) gained, which is considered to represent good value for money.
Conclusion: Improvements in glycemic control with insulin aspart as bolus therapy were projected to lead to decreased complication rates and improved quality-adjusted life expectancy compared to human insulin in the long-term treatment of patients with type 1 diabetes. Insulin aspart was associated with cost savings or would be considered cost-effective versus human insulin over patients' lifetimes in these nine European countries.

Difference between insulin aspart and human insulin

	Life expectancy (years)	Quality-adjusted life expectancy (QALYs)	Lifetime costs	ICER / Outcome
Austria	0.13	0.13	€ -193	Dominant
Denmark	0.09	0.09	DKK 399	DKK 4,215/ QALY
Finland	0.06	0.07	€ 285	€ 4,186/QALY
France	0.18	0.17	€ -309	Dominant
Germany	0.06	0.07	€ 563	€ 8,284/QALY
The Netherlands	0.07	0.08	€ -635	Dominant
Norway	0.09	0.09	NOK -1,021	Dominant
Spain	0.04	0.06	€ 1,202	€ 19,493/QALY
Sweden	0.09	0.09	SEK 2,612	SEK 27,601/ QALY

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Quality of care

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Cross-sectional investigation of treatment quality for international performance benchmarking: comparison of 14 largest German and Austrian diabetes centers in parameters of outcome and process quality

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Background and Aims: The creation of the databases for quality measurement purposes in Germany and Austria (FQSD, Forum Qualitätssicherung in Diabetes) was a key event in competitive benchmarking in diabetes care. We have been using FQSD system while steadily targeting quality improvement in care with means including ISO 9001 conformity (certification), development of quality management handbook and media (books and educational curricula) based on customer orientation with modular group education (modules: diabetes and functional insulin treatment FIT, metabolic syndrome and obesity, hypertension, hyperlipidaemia, pregnancy in diabetes, hypoglycaemia unawareness). In this joint project, German and Austrian blinded data were independently evaluated by FQSD statistician for center comparison in cross-sectional investigation of selected process and outcome quality indicators.
Materials and Methods: Selection criteria for voluntarily registered centers included biggest available numbers of individuals per centre assessed with annual basic information sheets acquired between April 1st, 2002 and July 1st, 2004, care for mainly adult outpatients with type 1 and type 2 diabetes and the availability of HbA_{1c} assay reference ranges (reference mean=100%) in order to assure the comparability.
Results: Seven German and seven Austrian centers with a total of 16403 diabetic individuals have been selected: all patients/type 1/type 2: n=16403/2335/13114, male 47/53/49%, age 60 ± 16/40 ± 15/65 ± 12, diabetes duration 10 ± 10/16 ± 13/10 ± 9yrs, relative HbA_{1c} 142 ± 32/148 ± 32/142 ± 31%, systolic blood pressure (BPsys) 138 ± 20/128 ± 17/141 ± 20, BPdia 80 ± 11/77 ± 10/80 ± 11 mmHg, total cholesterol 206 ± 49/201 ± 46/206 ± 48, LDLchol 119 ± 39/114 ± 35/120 ± 40 mg/dl, inpatients 16/6/19%, diabetes education 54/71/52%, annual eye exam 38/51/38%, foot exam 75/75/78%. We achieved the best positions for relative HbA_{1c} (type 1 diabetes 130%, type 2 diabetes 120%), the lowest systolic (123 ± 17 mmHg) in type 1, $p<0.001$, and diastolic BP (72 ± 10 mmHg), total (191 ± 33 mg/dl) and LDLchol (99 ± 28 mg/dl) in both diabetes types ($p<0.001$ vs the rest of the cohort) despite the longest diabetes duration in type 2 (16 ± 12yrs) and almost the longest one in type 1 patients (21 ± 12yrs). We have ranked best for percentage of structured education (95% of all our patients), for percentage of inpatients (0%) and the second best for percentage of annual foot and eye exam (94%).
Conclusion: The results of this independent evaluation of 14 largest German speaking diabetes centers in FQSD database show an excellent clinical efficiency of our outpatient modular group education system.

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Cultural, social and demographic factors that influence adherence to treatment guidelines in Latino patients with diabetes within a multicultural society

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Background and aims: Diabetes management is a challenge in multicultural societies. It is known that multiple medical, demographic, social and cultural factors influence diabetes care and outcomes and yet, very few programs address all these factors in routine clinical practice. In order to investigate the effects of some of these factors on patients' adherence to diabetes treatment guidelines, we conducted a cross-sectional study in patients with diabetes that attend the Latino Program of the Latino Diabetes Initiative at Joslin Diabetes Center in Boston. Latinos represent the largest and fastest growing minority in the US with one of the highest diabetes prevalence rates.
Materials and methods: We studied 92 patients who underwent the following evaluations: 1) A questionnaire that assesses aspects such as adherence to treatment recommendations, diabetes self care management, acculturation level, health beliefs, social and family support, employment, disease perception, depression, motivation to change, problem areas in diabetes (PAID questionnaire) and diabetes knowledge, among others. This questionnaire is based on previously reported and validated instruments. 2) a complete medical evaluation and 3) general blood chemistry and urine tests.
Results: 92 Hispanic/Latino patients with type 2 diabetes were included (59F/33M), age 53.8 ± 11.2 yrs (mean±SD), diabetes duration 11 ± 8.2 yrs (mean±SD). Patients were divided into a high adherence to treatment group (HAG) and low adherence to treatment group (LAG). The following variables were significantly higher in the HAG than in the LAG: Family support (72% Vs 33.3%, p=0.017), having a Spanish-speaking health care provider (76% Vs 41.7%, p=0.034), feeling comfortable with their health care provider (90% Vs 50%, p=0.011) and absence of depression (80% Vs 53.7%, p=0.014). When comparing patients with depression with those without it, the following variables were significantly higher in the depression group: Female gender (67.8% Vs 45.5%, p=0.047), unemployment (66.7% Vs 45.5%, p=0.037), lack of social support for diabetes care (76% Vs 52%, p=0.041), low adherence to medications (78.1% Vs 22%, p 0.014), perception of bad health (78% Vs 43%, p=0.001), lower diabetes knowledge (81.1% Vs 19%, p=0.043) and higher diabetes related distress as measured by the PAID score (40 Vs 24, p=0.001). Higher PAID scores were also found in patients with A1c levels higher than 7% when compared to those with A1c below 7% (35.3 Vs 19.23, p=0.007).
Conclusions: Multiple cultural, social and demographic factors are associated with adherence to diabetes treatment recommendations. In our population comprised by Latino patients living in a multicultural society such as that in the USA, the most important factors associated with high adherence to treatment recommendations were family support, having a provider that speaks the same language, good rapport with the health care provider and absence of depression. We have confirmed that depression is an important factor that influences diabetes treatment and is associated with several demographic and social characteristics. Taking into consideration all these factors appears to be important in designing and implementing culturally oriented patient care and education programs for people with diabetes living in a multicultural society.
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Quality of life of diabetic patients asssessed by the WHO 5-item well-being index

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Background and Aims: The aim of the present cross-sectional study was to evaluate the quality of life of diabetic patients by means of the WHO 5-item Well-Being Index (WHO-5).
Materials and Methods: 102 type 1 diabetic patients (mean age 39.2±14.1 years and mean duration of the disease 9.9±6.4 years) and 121 type 2 diabetic patients (mean age 58.9±19.7 years and mean duration of the disease 11.6±8.9 years), recruited at a university medical center, and 84 healthy controls (mean age 49.1±10.2 years) participated in the study. We administered a questionnaire to each individual to assess demographic characteristics, type and duration of diabetes, treatment, complications. To evaluate the quality of life we used the validated translated version of the WHO-5.
Results: Type 1 diabetic patients demonstrated significantly lower score ($p<0.05$) as compared to the healthy controls, while the difference between type 2 diabetic patients and the controls was not significant ($p>0.1$). There was a significant negative correlation between the WHO-5 index and the duration of diabetes ($r=-0.3981$, $p<0.05$) and the presence of diabetic retinopathy ($p=0.03$). No correlation was established between the well-being and patients' sex, age and kind of treatment. There was a significant difference in the well-being index between the patients who had attended a group diabetes education program as compared to the rest of the patients ($p<0.01$ for type 1 diabetic patients and $p=0.04$ for type 2 diabetic patients, respectively).
Conclusion: These results demonstrate that diabetes education has a crucial role in improving the well-being of patients with type 1 and type 2 diabetes. We consider that all patients with diabetes should be encouraged to attend a diabetes education program.

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Reduction of hospital stay and postoperative infections with an insulin protocol at a community heart hospital

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Background and Aims: Clinical trials have demonstrated the clinical benefits of tight glucose control with insulin in critically ill patients. We assessed the clinical benefits and hospital expenditures in patients admitted to a community heart hospital.
Materials and Methods: In April 2003, an insulin protocol was instituted at the hospital whereby all patients with glucose value >125 mg/dL were to receive insulin infusions then transitioned to a basal-bolus regimen of insulin glargine and lispro. Analysis of the hospital electronic database identified 2,470 patients (median age 65 years, 40% female, 80% Caucasian, 12% African American) with 3,047 admissions from February 2003 to March 2004. Overall, patients receiving insulin upon admission (n=1007) had an average length of stay (LOS) of 5.9 vs 2.6 days for those with no insulin (n=2040), an APACHE score of 14 vs 11, and a glucose level of 193 vs 142 mg/dL over the 1st 24 h of admission. To control for differences in duration of insulin therapy, we evaluated outcomes in the 614 admissions where insulin started from day 1 vs admissions where no insulin was given (n=2,040). Outcomes measures included total LOS, incidence of postoperative infections, adverse discharges to intensive care facilities, other hospitals or death, and total hospital charges. Normal and binomial random effects models were used to adjust for multiple admissions per patient, controlling for age, gender, race, APACHE II, AMI, CABG, discharge dispositions, total drug charges, glucose variability and number of glucose tests.
Results: Insulin therapy was associated with a 0.5-day reduction ($P<.0001$) in LOS independent of glucose variability; the LOS benefit increased with APACHE (interaction, $P=.001$) and reached 1 day ($P<.0001$) at APACHE of 23 points. Postoperative infections were 2-fold higher ($P<.0001$) in the no-insulin group. Adverse discharges increased more rapidly with APACHE for the no-insulin group in AMI and CABG patients (interactions, $P=.005$ and .029, respectively). There was no adjusted difference in hospital charges between admissions with and without insulin.
Conclusions: Insulin therapy may decrease length of hospital stay beyond glucose stabilization and reduce postoperative infections and adverse discharges at no additional hospital costs.
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Effectiveness of diet-and-exercise as initial therapy for type 2 diabetes in a large US population

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Background and Aims: Diet-and-exercise is increasingly the initial therapy for patients newly diagnosed with type 2 diabetes, but its success is little studied in real-world populations.
Material and Methods: Study subjects were drawn from the 2512 members of the Kaiser Permanente Northwest (a 450,000-member non-profit integrated health system in the USA) who were newly recorded as diagnosed with type 2 diabetes in 1999 or 2000, and who were continuously enrolled in the health system from diagnosis through the end of 2004 (follow-up 48–72 months). We selected the 1411 patients (56.2%) who had an initial therapy of diet and exercise, defined as no anti-diabetic drug treatment within the first three months of diagnosis. We observed them until an anti-hyperglycemic drug was initiated or until 31 December 2004. Subjects averaged 1.7 HbA_{1c} tests results/year.
Results: HbA_{1c} at diagnosis was below 7% in 54.1% of all subjects who attempted diet and exercise, and between 7 and 8% in another 28.4%. Overall mean HbA_{1c} at diagnosis was 7.1+/-1.3%. Younger patients had higher initial HbA1c's. After diagnosis, 43.2% of subjects (n=610) achieved an improvement of at least 0.5 percentage points of HbA_{1c}. Improvements of this magnitude were less frequent when initial HbA_{1c} was already <7%. Most patients with initial HbA1c>8% (54.7%) never reached an HbA_{1c} <7% and, when reached, this goal tended to be attained within 12 months. However, those who did succeed experienced very large mean reductions in HbA_{1c} (-0.86 and -3.76 percentage points). Among the 610 patients whose HbA_{1c} improved at least 0.5%, mean months until best- HbA_{1c} ranged from 17.7 to 35.3. The best HbA_{1c} averaged less than 7% for all initial-HbA1c strata, among patients who improved at least 0.5%. Overall, however, about half of all subjects (50.4%) ended up initiating anti-hyperglycemic drug therapy before the end of follow-up. Patients with initial HbA_{1c} <7% were about half as likely to start a drug (34.3%) as other patients (65.6, 71.1 and 74.3%). Among all 1411 subjects, by the end of observation (study end or drug initiation), the mean change in HbA1c from baseline was an increase of 0.16 percentage points.
Conclusions: In this population, more than half of patients with newly diagnosed type 2 diabetes now attempt extended non-drug therapy. Most have HbA_{1c} <7% at diagnosis. Of those with higher initial HbA_{1c}, about two-fifths improve to <7% within the first post-diagnostic year. To achieve an HbA_{1c} goal below 7% in the remaining three-fifths, either behavior-change therapy should be intensified or an anti-hyperglycemic agent should be started more quickly than is currently done.

Mean Glycemic Control Success, by HbA_{1c} at Diagnosis

Initial HbA_{1c} Category	<7% (N=763)	7–7.9% (N=401)	8–8.9% (N=142)	>=9% (N=105)	All (N=1411)
Mean change in *HbA_{1c}* at end of obsservation	0.43	0.27	–0.44	–1.35	0.16
Percent whose *HbA_{1c}* improved > 0.5	30.8%	54.9%	64.8%	60.0%	43.2%
Max change in *HbA_{1c}*, if improved >0.5	–0.86	–1.23	–1.86	–3.76	–1.44
Mean change in *HbA_{1c}* if not improved >0.5	0.79	1.01	0.69	0.40	0.81
Number (%) Achieving *HbA_{1c}* < 7.0%, by Year When This First Occurred …					
First Year *(post diagnosis)*	––	172 (42.9%)	53 (37.3%)	33 (31.4%)	258 (39.8%)
Second Year	––	42 (10.5%)	11 (7.8%)	5 (4.8%)	58 (9.0%)
Third Year	––	19 (4.7%)	2 (1.4%)	2 (1.9%)	23 (3.5%)
Fourth thru Sixth Year	––	13 (3.2%)	2 (1.4%)	4 (3.8%)	19 (2.9%)
Never (after mean follow-up of 17.7 months)	––	155 (38.7%)	74 (52.1%)	61 (58.1%)	290 (44.8%)
Months until first anti-diabetic drug	31.1	24.8	21.0	20.2	26.1

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Thirteen years of diabetes management in a municipal hospital system

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Since poor glycemic control is a particular problem for ethnic minority patients in urban environments, the Grady Diabetes Clinic has attempted to improve outcomes by emphasizing both aggressive management by providers and regular assessment of management. To evaluate the impact of this quality improvement program, we reviewed care in this setting between 4/1/1991 and 12/31/2004. 10,874 patients with type 2 diabetes and first visits to the clinic had average age 52 yrs, BMI 33 kg/m^2, and diabetes duration 5.2 yrs; 61% were female, and 89% were African American. HbA_{1c} at presentation fell from 9.0% in 1991 to 8.3% in 2004 ($p<0.0001$). Provider management during the first year of followup care improved significantly over time: intensification of therapy when indicated increased from 21% of the time in 1991 to 61% in 2004, and the amount of intensification increased from 17% of our current recommendations in 1991 to 84% in 2004 (both $p<0.0001$ for trend). This improvement in provider behavior was associated with lower HbA_{1c} levels in the 3,405 patients who returned for followup visits after 1 year of care; HbA_{1c} at the end of the year fell from 8.1% in 1991 to 7.2% in 2004 ($p<0.0001$ for trend). Patient adherence to medications (mean ~88% of recommended; 93% in 1991 to 69% in 2004) and appointments (mean 4.0 per year; 4.6 in 1991 to 3.0 in 2004) was relatively high throughout the 12-year period but showed a significant downward trend over time ($p<0.0001$). In multivariable linear regression analysis, adjusting for age, sex, BMI, race, diabetes duration, year of presentation, initial HbA_{1c}, and patient adherence, the magnitude of provider intensification was significantly associated with a greater fall in HbA_{1c} over 1 year of care $p<0.0001$.
Conclusions: In a municipal hospital diabetes clinic, self-assessment strategies improved provider behavior significantly over 13 years, in association with better glycemic control – close to ADA goals. Translating these

approaches to enhance diabetes care across the U.S. may require a similar emphasis on evaluation and improvement of management by providers.

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Diabetes mellitus in eight new EU accessing countries: basic description of quality of care

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Background and Aims: To perform a baseline evaluation of diabetes care in the Czech Republic, Estonia, Hungary, Lithuania, Latvia, Poland, Slovakia and Slovenia.
Subjects and methods: Patients were randomly enrolled. Questionnaires for each patient were completed by a diabetologist or endocrinologist. Questions included data about age, sex, duration of diabetes, type of diabetes, type of treatment, metabolic control (HbA_{1c}), lipid data (total cholesterol, LDL cholesterol, triglycerides and HDL cholesterol), blood pressure (BP) and data about short and long term complications. Questionnaires were analyzed centrally for each country for all patients, and stratified for type1, type2 and other types of diabetes.
Results: Data on 11,085 individuals were analyzed (mean age: 56.21 yrs, females: 51.97%, type 1 diabetes (T1DM), 22.85%, type 2 diabetes (T2DM), 75.31%; mean time from diagnosis, 10.95 yrs). We found, that the percentage of patients with an HbA_{1c} within target (<6.5%) was 13.13% in T1DM and 21.44% in T2DM; that targets for total cholesterol levels (<4.5 mmol/L) were achieved in T1DM only in 36.58% and in T2DM in 19.67%; targets for triglyceride levels (<1.7 mmol/L) were achieved in T1DM in 78.01%, in T2DM in 43.54%. The targets for HDL cholesterol (> 1.1 mmol/L) were achieved in T1DM 81.19% and in T2DM 59.97%; and for LDL cholesterol (< 2.5 mmol/L) in T1DM 36.08% and in T2DM 22.38%, respectively. The targets for BP (lower than 130/80 mm Hg) were achieved in 41.92% of T1DMand in 8.62% of T2DM. The prevalence of severe hypoglycaemia - within the last six months- was 11.61% (T1DM) and 2.14% (T2DM). The prevalence of ketoacidotic coma had a range of 0.27% to 6.56% across the eight countries. We found that the prevalence of blindness ranged in countries from 0,15% to 1,31%, and of diabetic nephropathy from 19.09% to 41.67%. Results of macrovascular complications are summarized in Table 1.
Conclusion:
The data shows the current status of the quality of care and the potential fields of improvement. Furthermore, significant differences were found between the individual countries. The results should be analyzed in more detail as a basis for indicating required resources for national health services.

Table 1. Prevalence of macrovascular complications

Type 1	MI	2.68%
	Other forms of CHD	6.33%
	Stroke or TIA	1.36%
	PAD	6.57%
Type 2	MI	12.42%
	Other forms of CHD	31.16%
	Stroke or TIA	7.40%
	PAD	16.54%

Abbreviations: MI=myocardial infarction; CHD=coronary heart disease; TIA=transient ischemic attack; PAD=peripheral arterial disease

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Discrete event simulation of diabetes and its complications

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Background and Aims: Given the long-term, complex nature of the disease, models are commonly used to estimate the health and economic implications of diabetes management strategies. Recent ADA guidelines for computer modeling define criteria for such models: transparency, validation, and uncertainty assessment. Most commonly, Markov representations are used to model the disease as mutually exclusive "health states" and transitions among these. Even with many parallel sub-models for each complication type, the multiple resulting states create immense complexity. Further, the assumptions required and inflexibility of these models limit their ability to adequately reflect the diabetes disease process; transparency is also impaired. Discrete event simulation is a much more flexible, transparent technique but it has not been used in diabetes. The aim of this study was to develop a discrete event simulation of the long-term impact of managing risk factors and attaining glycemic control in patients with diabetes.
Materials and Methods: Individual hypothetical patients are created by assigning each patient characteristics and then "cloning" them to study multiple scenarios of care; each clone receives one of the treatments studied. Patients begin at home and visit a physician periodically, where lab and clinical values are updated. Patients may develop complications (and require hospitalization), die or change treatment. Times for each event are sampled from failure time distributions specified by the individual's time-dependent risk profile based on updated personal characteristics, treatment and local practice patterns. Published studies were used to derive risk equations for death, stroke, myocardial infarction, progression of nephropathy, retinopathy, and neuropathy, which are applied simultaneously. Resources (e. g., hospitalizations, physician visits) are explicitly simulated. The model is programmed in a graphical interface (ARENA®) that ensures transparency of design, inputs and calculations. First-, second-order and structural uncertainty are readily assessed. Data from a specific population can also be used as input for patient characteristics.
Results: Simulations of thousands of cloned patients, each characterized by more than 100 different attributes (e. g., age, sex, smoking status, glycemia, lipids), take less than 5 minutes, providing more than 100 results over time.
Conclusion: Discrete event simulation permits realistic modeling of diabetes, meeting ADA guidelines while avoiding over-simplification of diabetes management.

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Will protocollized, software supported diabetes care improve the cardiovascular risk profile of patients with type 2 diabetes in general practice in one year?

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Introduction: Patients with type 2 diabetes (DM2) have a two to five fold increased risk for cardiovascular disease compared to non diabetics. Quality of care for DM2 patients can be measured by calculating their absolute risk of developing a cardiovascular disease. Implementing high standard of diabetes care appears to be difficult. The use of diabetes care programs with repetitive collection of data, scheduled invitation of patients and medical decision sustaining software such as the Diabetic Care Protocol (DCP), developed by D4H (Diagnosis 4 Health), may be helpful in improving diabetes care indicated by a reduced cardiovascular risk profile.
Objective: To investigate the change in cardiovascular risk profile for patients with DM2 after implementation of the DCP in general practice.
Method: General practitioners (GPs) in the Netherlands using the DCP were asked to participate in a before-after trial for one year. Organisation of diabetes care was optimized by rigorously delegating routine tasks to specially trained paramedics who used software that supported management and medical decision making. Quality and continuity were monitored through a dynamic, real-life clinical database. This enabled both feedback and benchmarking for the GP every three months.
Outcome measure: The mean risk for developing a cardiovascular event within 10 years was calculated using the UKPDS risk-engine. Reduction of cardiovascular risk profile was investigated after one year. (paired t-tests).
A multiple regression analysis with dependent variable HbA_{1c}T12 was made using the following variables at T0: Age, HbA_{1c}, systolic BP, diastolic BP, total cholesterol, BMI, well-being, familiar diabetes, smoking pack-years, race and duration of diabetes.
Results: A total of 4511 patients (66 general practices) were included. After one year the mean risk for developing a cardiovascular event within 10 years was reduced from 4.5% to 4.0%. ($p<0.05$). Risk profile: mean HbA1c decreased from 7.0% to 6.8%, Systolic BP from 148 mmHg to 143 mmHg, Diastolic BP from 83 mmHg to 80 mmHg and total cholesterol from 5.2 mmol/l to 4.8 mmol/l. (all measurements $p<0.05$). After one year 66% of the patients reached HbA_{1c} <7% (compared to 59% at baseline), 62%

reached RR<150/85 mmHg (52%) and 61% reached cholesterol <5 mmol/l (47%). (all measurements p<0.05). Multiple regression analysis showed that HbA1cT0 was correlated to HbA1cT12. (p<0,05).
Conclusion: Delegating routine tasks in diabetes care using special software supporting management and medical decision making reduced cardiovascular risk-score by 11%. All patients seemed to benefit from this program and those with poor glycaemic control most. However, since unknown variation exists between the individual GPs and their management of diabetes care further randomised clinical investigation is necessary.

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Inflammation, diabetes and retinopathy

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Reduced antioxidant response of retinal and endothelial cells in response to chronic oscillating glucose levels

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Background and aims: To examine the antioxidant response of two retinal cell types and one endothelial cell type in response to AGE and constant or oscillating glucose high glucose.
Materials and Methods: Human ARPE-19 retinal pigment epithelial cells, human retinal pericytes and human endothelial cells (HUVECs) in culture were exposed for 14 days to high constant or oscillating glucose. Activities of total superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) were measured. Levels of 8-OH-dG and 3-nitrotyrosine (NT) were assayed using ELISA and western blot.
Results: High constant glucose exposure resulted in significant increases in SOD activity with a small effect on GPx and catalase levels in all three cell types. Oscillating glucose, on the other hand, resulted in no induction of the activity of SOD, GPx, or catalase in the ARPE-19 cells and pericytes and resulted in attenuated SOD and GPx activity in HUVECs as compared to high glucose. In cells exposed to oscillating glucose, levels of both 8-OH-dG and NT were increased as compared to either high constant or normal glucose.
Conclusions: In one endothelial and two retinal cell types, chronic oscillating glucose exposure resulted in decreased antioxidant response and increased levels of markers of reactive oxygen (8-OH-dG) and reactive nitrogen (NT) species as compared with chronic high glucose levels. This implies that the increased oxidative stress associated with the postprandial state in diabetes might be due in part to a reduced antioxidant response and that a balance between reactive oxygen species production and detoxification exists in the diabetic retina.

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Evaluation of the inflammation events in the pathogenesis of diabetic retinopathy. Possible role of hypertension in the exacerbation of the retinopathy

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Background and Aims: Arterial hypertension is the main secondary factor associated with diabetic retinopathy (DR). However, the cellular mechanism of interaction between hyperglycemia and hypertension in the development of DM is poorly understood. Therefore, the aim of the present study was to investigate the effects of hyperglycemia and hypertension in the early inflammatory phenomena in retina in a model of genetic hypertension and experimental diabetes.
Materials and Methods: Diabetes was induced in male 4 and 12 weeks old spontaneously hypertensive rats (SHR) and and their normotensive control Wystar Kyoto (WKY) rats by administration of streptozotocin (60 mg/kg, i.v); animals presenting blood glucose ≥270 mg/dl were included into the study and sacrified 20 days after the induction of DM. The expressions of NF-kB, VEGF and ICAM-1 were evaluated by Western blot analysis.
Results: In the 4 week-old rats, the retinal expression of VEGF was significantly higher in diabetic SHR compared with their normotensive controls (1,06 ± 0,08 vs 1,24 ± 0,18 and 2,01 ± 0,45 vs 2,79 ± 0,88, respectively for control WKY vs diabetic WKY and control SHR vs diabetic SHR, p<0,0001,). Among 12 week-old animals, there was a significant increasing in the retinal expression of VEGF (1,43 ± 0,20 vs 1,08 ± 0,27 and 1,46 ± 0,31 vs 2,51 ± 0,59, respectively for control WKY vs diabetic WKY and control SHR vs diabetic SHR, p<0,0001), ICAM-1 (p=0,007) and the total NF-kB in the retinal tissue in diabetic SHR compared with their normotensive controls (4,60 ± 0,83 vs 6,21 ± 1,49 and 2,60 ± 1,35 vs 5,37 ± 1,49, respectively for control WKY vs diabetic WKY and control SHR vs diabetic SHR, p=0.03).

Conclusion: The induction of diabetes in SHR rats promotes increase in VEGF, ICAM-1 and NF-kB in retinal tissue earlier in the hypertensive rats. These data suggest a mechanism by which hyperglycemia and hypertension interacts in the development of DR.
Support: Fapesp, CNPq, Capes

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Endothelial survival factors, but not pericyte coverage of retinal capillaries determine responsiveness to vasoregression in the retina
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Background and Aims: Pericyte loss and capillary regression are characteristic for incipient diabetic retinopathy. Pericyte recruitment is involved in vessel maturation, and ligand-receptor systems contributing to pericyte recruitment are survival factors for endothelial cells in pericyte-free in-vitro systems.We studied pericyte recruitment in relation to the susceptibility towards hyperoxia-induced vascular regression, addressing the important question of pericyte function in the angiogenic retina. **Materials and Methods**: For studying pericyte recruitment to the developing retina, we used the pericyte reporter X-LacZ mouse and retinal morphometry. The angiogenic response to hypoxia was determined in the mouse model of retinopathy of prematurity. The expression patterns of VEGF, angiopoietins, and PDGF-B were determined by PCR and immunoblotting.
Results: Pericytes were found in close proximity to vessels, both, during formation of the superficial and the deep capillary layers. Hyperoxia-induced vessel regression was only possible before the deep capillary layer had formed (i.e. postnatal day 8). This resistance was associated with a combined upregulation of angiopoietin-1 and PDGF-B, while VEGF was unchanged during the transition from a susceptible to a resistant capillary network. Inhibition of Tie2 function either by soluble Tie2 or by a sulindac analogon, an inhibitor of Tie2 phosphorylation, resensitized the retina to increased neovascularization by inhibiting the formation of the deep capillary network without affecting pericyte recruitment.
Conclusion: Our data indicate that vessel resistance to regressive stimuli is mediated through the upregulation of endothelial survival factors induced by the completion of the retinal vessel network, and is independent of pericyte coverage.

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Vitreous esRAGE, a decoy receptor for AGE, is associated with the progression of diabetic retinopathy
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Background and Aims: Advanced glycation endproducts (AGE) engagement of the receptor for AGE (RAGE) participates in the development of diabetic vascular complications. A novel splice variant cording for an endogenous decoy form of RAGE (esRAGE) was recently identified and thought to modify the RAGE-associated vascular diseases. In this study, we investigated a relationship between vitreous esRAGE levels and the progression of diabetic retinopathy.
Materials and Methods: Thirty-three samples of vitreous fluid were obtained during vitrectomy operation with informed consent from 4 T1DM and 29 T2DM patients (male:female, 20:13; average age, 52 ± 14 years; Retinopathy, background:proliferative, 7:26; ACR<12 mg/gCr: 12<ACR <300:ACR>300 mg/gCr, 5:9:19). esRAGE levels in vitreous fluid and serum were measured by a newly developed sandwich ELISA system (sensitivity>0.017 ng/ml, Daiichi Fine Chemical, Co, Ltd).
Results: The esRAGE level of vitreous fluid in patients with proliferative retinopathy was significantly higher than that in patients with background retinopathy (31 ± 14 pg/ml vs. 25 ± 5.0 pg/ml, $p<0.05$), but there is no significant difference in the serum esRAGE level between two groups ($p=0.14$). On the contrary, esRAGE in serum was correlated to ACR levels ($r=0.607$, $p<0.0005$).
Conclusion: In summary, we first detected an esRAGE in vitreous fluid from the patients with diabetes. The results suggest that vitreous esRAGE could be increased by concomitant intraocular vascular damages and might play a role in the progression of diabetic retinopathy.

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New-onset diabetes mellitus after transplantation
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Background and Aims: New-Onset Diabetes Mellitus (NODM) is a serious complication of transplantation, associated with a decrease in survival of graft and patient. The purpose of this study was to analyze the incidence of abnormal glucose regulation, its impact on survival of graft and patient, and identify the risk factors for its development in patients undergoing renal transplantation in our hospital.
Materials and Methods: 920 non-diabetic patients who underwent renal transplantation between February 1980 and October 2004 in our hospital, whose graft was functioning after one month of follow-up, were reviewed retrospectively. 63% male, 42.9 ± 12.3 (range, 14 to 71) years old at time of transplantation. Their follow-up included fasting plasma glucose monitoring 1, 3, and 6 months after transplantation and yearly thereafter. Abnormal glucose regulation was analyzed following 1997 ADA criteria. All data were statistically analyzed using the SPSS/PC 10.0.1 program, including Kaplan-Meyer curves for survival and logistic regression analysis for risk factors.
Results: 95 patients (10.3%) showed impaired fasting glucose (IFG) and 118 (12.8%) were found to have DM at some point during follow-up. Of these, 46.3% were treated with diet, 23.7% with oral agent and 30% with insulin. 66 (55.9%) of the patients with NODM demonstrated a transient DM. 25 patients (21.2%) of non-transient DM required insulin therapy. The presence of IFG significantly affected survival of graft and patient. Risk factors for the development of IFG or DM were shown to be initial body weight (68.7 ± 13.0 kg vs. 65.4 ± 11.6 kg) and age (45.7 ± 11.1 years vs. 43.1 ± 12.3 years). The remaining factors analyzed (cause of death of the donor, presence of acute tubular necrosis, acute rejection, and HCV infection) were not proven to be independent risk factors for the development of NODM, neither was a relationship shown with immunosuppressive therapy: cyclosporine, tacrolimus or non-calcineurin inhibitor agents.
Conclusion: 1. NODM after kidney transplantation demonstrates high incidence. 2. IFG influences negatively and significantly the survival of graft and patient. 3. Risk factors proven to be independent were age and body weight of the recipient; HCV infection and initial therapy with tacrolimus were not.

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Hepatic glucose metabolism in renal transplant patients treated with cyclosporine
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Background and Aims: Renal transplantation is available to a growing number of patients with kidney failure; hence there is increased interest in the etiology and treatment of post-transplant diabetes mellitus (PTDM): a frequent but poorly understood complication. Immunosuppressant therapy (IsT) may play a key role in PTDM development. Of the IsT drugs in current use, cyclosporine (CsA) is considered one of the least diabetogenic. Nevertheless, a significant number of kidney transplant recipients treated with CsA develop PTDM. Unlike some other IsT agents, CsA does not inhibit insulin secretion. However, plasma triglycerides are elevated suggesting a loss of lipid homeostasis and increased availability of fat for hepatic oxidation. Hepatic gluconeogenesis (GNG) is highly sensitive to fatty acids availability and might therefore be increased in this setting. To determine the effects of CsA on hepatic glucose metabolism, GNG contribution to fasting glucose production (GP) was quantified by the ^2H_2O method in non-diabetic kidney transplant patients receiving CsA and in a control group of healthy study subjects without ongoing IsT.
Materials and Methods: At 5 and 7 h fast the study subjects were given a 35% ^2H_2O to attain body water enrichment of ~0.5%. Paracetamol (1000 mg) was also taken at 7 h. Urine was collected during 8–10 h and 10–12 h of fasting. Blood samples were drawn at 12 h. Urinary paracetamol

glucuronide was isolated and derivatized to monoacetone glucose (MAG) and the ratio of deuterium enrichment in positions 5 and 2 (D5/D2) quantified by ^{2}H NMR Spectroscopy.
Results: ^{2}H NMR spectra of MAG had signal-to-noise ratios of at least 20:1 allowing confident quantification of D5/D2 from the relative intensities of the ^{2}H NMR signals for these positional enrichments. The fractional contribution of GNG to endogenous GP was increased in the post-transplant patients relative to controls and no difference was observed between the 8–10 h and 10–12 h fast within the same group (Table 1).

Table 1. Study subjects and characterization of hepatic glucose metabolism

	Kidney post-transplant patients[+]		Controls	
Gender (male/female)	7/0		3/3	
Age, yr	45 ± 2*		34 ± 3	
BMI, %	24.1 ± 1.0		24.7 ± 1.6	
Fat, %	9.8 ± 3.5		11.2 ± 3.4	
Glucose, mg/dL	85 ± 2		84 ± 4	
Insulin, μUi/mL	17.3 ± 9.2		6.1 ± 2.0	
Cholesterol, mg/dL	218 ± 17		184 ± 21	
Triglycerides, mg/dL	166 ± 25		98 ± 29	
	8–10 h fast	**10–12 h fast**	**8–10 h fast**	**10–12 h fast**
Percentage of GP from Gluconeogenesis, %	60 ± 2**	59 ± 2*	49 ± 4	50 ± 2

[+] Duration of transplant= 6 ± 2 yr. Data are expressed as mean±SEM. Statistical significance was determined by using the Student's *t* test; * $p<0.01$;** $p<0.05$

Conclusion: Our preliminary results suggest that CsA IsT is associated with a modest but significant increase in the fractional contribution of GNG to fasting endogenous GP. These changes are accompanied by a tendency for increased plasma triglyceride and cholesterol levels. These features have similarities to the disturbed glucose homeostasis of type 2 diabetes where elevated rates of GNG and endogenous GP contribute to the development of fasting hyperglycemia. We speculate that the increase in plasma lipid levels and the gluconeogenic contribution to fasting GP associated with CsA IsT may represent the initial stages of a non-insulin dependent type of PTDM.
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Retinopathy, risk factors

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G protein β3 subunit polymorphism as a susceptibility factor for diabetic retinopathy
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Background and Aims: Although glycemic control influences much in their development, chronic diabetic complications such as diabetic retinopathy are still complex multifactorial disorders, resulting from an interaction of genetic as well as environmental etiologies. Several genetic variants have been shown to increase the risk of diabetic retinopathy (DR). However, the genes that underlie its genetic susceptibility remain to be identified. Genes encoding elements of the G-protein system have been reported to be candidate genes in hypertension and obesity. Since insulin uses G-sensitive mechanisms to enhance tissue glucose uptake and vasodilatation, the G protein β3 subunit (GNB3) gene at chromosome 12p13 may be a candidate gene of diabetes itself or diabetic microvascular complications. Therefore, we wanted to investigate whether the polymorphisms of the GNB3 gene may contribute to the independent susceptibility of the DR irrespective of their clinical subtype in Korea.
Materials and Methods: The study population comprised 252 non-hypertensive T1D patients (121 men, 131 women), 140 non-hypertensive T2D patients (72 men, 68 women) without retinopathy, and 152 healthy controls (82 men, 70 women). Fifty-seven T2D patients with proliferative diabetic retinopathy (PDR; 29 men, 28 women) and 121 T2D patients with background diabetic retinopathy (BDR; 64 men, 57 women) were also studied. Separate 148 non-diabetic subjects either had hypertension or had taken antihypertensive drugs (77 men, 71 women) were also recruited. Genotypes of SNPs were determined with a fluorescence-based allele-specific DNA primer-probe assay system.
Results: As expected, the TT genotype of GNB3 C825T had a susceptible influence for the risk of hypertension with the OR of 5.93 (95%CI: 2.25–16.51) ($p<10^{-4}$). However, the genotype distribution of the controls did not differ either from that of non-hypertensive T1D, T2D, or BDR. Overweight and obesity were not associated with the T825 variant in either the experimental subjects or the controls. Interestingly, compared with the normal controls, the TT genotype was decreased in patients with PDR (OR=3.41, $p<0.05$).
Conclusion: In our population, the T825 variant of the GNB3 gene was not associated with diabetes, nor with overweight and obesity, but was associated with hypertension and PDR. The GNB3 polymorphism, which will influence the development of hypertension may accelerate the development of PDR. Further prospective study will be needed for the elucidation of the possible role of GNB3 polymorphism in the development of this microvascular complication.

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Risk factors for microangiopathy in type 1 diabetic patients on functional intensive insulin therapy from the onset of the disease
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Background and Aims: The DCCT and its follow up EDIC study demonstrated that improvement of metabolic control and intensive insulin therapy reduces the risk of development and progression of late diabetic complications. The aim of this study was to assess the incidence and predictors of nephropathy and retinopathy in type 1 diabetic patients treated with intensive functional insulin therapy (IFIT) from the onset of the disease.
Materials and Methods: 100 patients with newly diagnosed type 1 diabetes (43 female, 57 male, aged 24.1 ± 6.1 years) were recruited to the study (between years 1994–1999). All subjects underwent a five-day teaching programme providing the skills in IFIT. We assessed at baseline and every 1 year: knowledge about diabetes and IFIT, number of hypoglycaemic episodes, quality of life, eye fundus, urine albumin excretion, glycaemic control, lipid profile, blood pressure and serum concentration of C peptide, hsCRP and other inflammatory markers (ICAM-1, VEGF, TNFα).
Results: The mean follow-up was 6.1 ± 1.6 years. In the group of 18 (20%) subjects with diabetic nephropathy we noticed higher values of: HbA1c

(9.0 ± 1.8 vs 8.0 ± 1.3, p=0.04), hsCRP (4.87 ± 4.91 vs 1.81 ± 1.89, p=0.02), triglycerides (132.89 ± 89.35 vs 88.05 ± 37.32, p=0.01), LDL-cholesterol (140.61 ± 41.89 vs 116.0 ± 34.32, p=0.01) and VEGF (376.62 ± 216.26 vs 250.68 ± 130.67, p=0.02). Moreover, we observed relationship between the development of nephropathy and high levels of hsCRP (OR=4.40; 95%CI: 1.15–16.86, p=0.047), LDL-cholesterol (OR=4.61; 95%CI: 1.33–15.97, p=0.01), diastolic blood pressure (OR=11.43; 95%CI: 3.16–41.36, p=0.0002) and BMI (OR=4.50; 95%CI: 1.42–14.22, p=0.013). Patients with retinopathy, compared to subjects without retinopathy, had higher: FPG (13.5 ± 4.1 vs 10.1 ± 3.6 mmol/l, p=0.0018), HbA1c (8.8 ± 1.3 vs 8.1 ± 1.4, p=0.04), SBP (128.1 ± 16.6 vs 119.8 ± 14.0 mmHg, p=0.04) and sICAM-1 (290.9 ± 100.0 vs 237 ± 12 ± 53.44 ng/ml, p=0.03). The risk of retinopathy was associated with low diabetic knowledge (OR=0.13; 95%CI: 0.02-0.93, p=0.02), higher SBP (OR=3.75; 95%CI: 1.16–12.19, p=0.03) and higher DBP (OR=7.43; 95%CI: 2.11–26.15, p=0.002).

Conclusion: Intensive functional insulin therapy improves quality of life in patients with type 1 diabetes, but does not prevent the development of diabetic nephropathy and retinopathy. The obtained results confirm the important role of metabolic control, blood pressure, inflammation and patients' knowledge in the pathogenesis of late diabetic complications.

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Type 1 diabetics with fair glycaemic control through 18 years have minimal retinopathy

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Background and Aims: Diabetic retinopathy is a major complication of diabetes mellitus. We assessed the association between 18 years glycemic control (mean HbA1c) and diabetic retinopathy in type 1 patients having used intensified insulin treatment with multiple injections or insulin pumps for 14–18 years.

Materials and Methods: Retinopathy at 18 years follow-up was evaluated by colour photograhies of the retinas. Micro aneurysms (Ma) and haemorrhages (He)were counted as red spots, and were assessed by an ophthalmologist who had no knowledge about the patients' glycaemic status. Laser treatment was also registered. Glycaemic status was evaluated yearly during 18 years using HbA_{1c} measurements.

Results: 39 patients were followed during 18 years. Mean age was 43 (35–58) years. Mean duration of type 1 diabetes was 30 (23–39) years and mean age at diagnosis was 12 years (5–21). Mean HbA1c was 8.2% (6.6–11.3). Mean number of red spots for each eye was 11.2 (0–76). Number of red spots was significantly associated with mean HbA1c during 18 years (r=0.471, p=0.002). Having hard exudates, age, duration of disease, microalbuminuria, BMI, smoking and total cholesterol value was not significantly associated with the number of Ma/He. We did analysis for tertiles of HbA_{1c} and the two groups with the lowest HbA1c did not have any significant difference in number of red spots. When comparing means below and above the third tertile for HbA_{1c} limit (8.4%); patients with HbA1c below or like 8,4% had a mean of 5.6 Ma/He and patients with HbA_{1c} above 8.4% had a mean of 20 Ma/He (p=0.003). 26.1% of the patients with HbA_{1c} under or like 8.4% had 0 red spots and 30.4% had 1,2 or 3 red spots, 43.5% had at least 4 red spots. Whereas, in patients with HbA_{1c} above 8.4%, 100% had at least 4 red spots. In the group with HbA_{1c} under or equal to 8.4% 1/24 had received laser treatment compared to 9/15 in the higher HbA1c group (p<0.001).

Conclusion: At 18 years follow-up these patients with type 1 diabetes on intensive insulin treatment had mostly minor retinal changes, most of which could be characterized as mild non proliferative diabetic retinopathy. However, the number of microaneurysms and retinal haemorrhages were significantly associated to mean HbA_{1c} during 18 years. Clinically, the importance of good glycaemic control over time was also underlined by a much higher need for laser treatment in the higher HbA_{1c} group.

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Incidence of proliferative retinopathy in relation to the age at onset of type 1 diabetes

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Background: Diabetic retinopathy is the most important cause of blindness in developed countries.

After 20 years of diabetes almost all type 1 and 60% of type 2 diabetic patients have some degree of diabetic retinopathy. Severe visual loss threatens 5–10% of all patients. It has been suggested that the incidence of diabetic retinopathy is influenced by the age at onset of diabetes. However, the data are not clear in all respects.

Aim: To study variables associated with proliferative diabetic retinopathy (PDR) and to assess whether age at onset of diabetes influences the incidence of PDR.

Patients and methods: As a part of the ongoing FinnDiane Study we have so far collected data on diabetic retinopathy from 1704 patients (888M/816F) with 26000 pages of ophthalmic records and 10 241 separate fundus photographs scanned at 300 dpi from 794 patients. The first 500 completely classified patients are used in the present analysis. Images are graded with a modified scale based on the ETDRS-grading by an ophthalmologist unaware of the demographic data and the presence or absence of other complications of the patients. A subset of the images is graded by an additional ophthalmologist in order to control for the interobserver error.

Results: 165 of the patients (33%) had PDR. All variables that in univariate analysis were associated with PDR (male sex, overt diabetic nephropathy, age, duration of diabetes, age at onset of diabetes, blood pressure and HbA_{1c}) were included in a multiple logistic regression analysis. In this analysis, only HbA_{1c}, duration of diabetes and the presence of overt diabetic nephropathy remained associated with PDR (r^2=0.437). When patients were divided into three groups based on age at onset of diabetes (0–5 years, 5–15 years, 15–40 years) the prevalence of PDR in the 0–5 age group was 34.8% (23/66), in the 5–15 age group 39.0% (99/253), and in the 15–20 age group 23.7% (43/181)(p<0.001). The duration to PDR was determined for the incident cases. The average duration to PDR was 23.5 ± 6.0 years in the 0–5 age group, 19.4 ± 7.0 years in the 5–15 age group, and 21.7 ± 7.5 years in the 15–40 age group (p= 0.02).

Conclusion: In our preliminary analysis, patients with an age at onset of diabetes below 5 years seem to develop proliferative retinopathy later than those with a higher age at onset. Proliferative retinopathy is associated with worse glycemic control, longer diabetes duration and overt diabetic nephropathy.

Support: Silmasaatio

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Retinopathy and nephropathy are the most prevalent complications among diabetic subjects in Bangladesh: a ten-years follow-up study

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Background and Aims: To determine the types and prevalence of complications among the diabetic subjects after ten-years follow up. To assess how much of the glycemic control was maintained during the period.

Materials and Methods: The study was conducted at BIRDEM, a national referral center for diabetes. The center registered 221,116 patients up to 31 December 2000. In each working day, BIRDEM registers more than 70 referred patients and about 1200 registered patients attend the center for regular follow up. We conducted the study in all working days from January to December 1995 at BIRDEM outpatient. We included all subjects with a unique Registration Number given from January 1980 to December 1985. Thus, we could investigate a historical cohort of 625 diabetic subjects (M/F: 300/325) with at least ten-years follow up. We retrieved the clinical and biochemical information (weight, blood pressure, condition of foot, skin, eye [fundoscopy], heart [ECG], blood glucose, lipids, creatinine, urea, electrolytes etc.) from the follow-up visits recorded in the guidebook owned by the subjects and also from BIRDEM registry.

Results: After ten-years of follow-up, the prevalence of foot ulcer, stroke and coronary heart disease (CHD) was 2.9, 3.4 and 15.0%, respectively. Almost all complications were found to increase at the end of 10-years than at 0-year and 5-year follow-up. Thus, the prevalence of CHD was found 3.5% during registration, which increased to 5.1 and 15%, respectively,

after 5 and 10 years of registration. Similarly, the prevalence of nephropathy was found to increase from 16.5% after 5-year to 20.6% after 10-years. Very high increase was observed in retinopathy, which increased from 12.3% at registration to 23.3% and 33.8% after 5 and 10 years follow-up, respectively. The mean (SD) blood glucose (2 h post-meal) was also found to increase from 9.3 (2.3) mmol/l at registration to 11.7 (2.4) mmol/l after 10-years follow-up ($p<0.02$).
Conclusion: There was a predominance of microvascular complications over macrovascular events in the diabetic subjects after a long-term follow-up. Thus, retinopathy and nephropathy was much higher than CHD and stroke. Possibly, those who developed CHD and stroke at early stage were lost to follow up owing either to disabilities or to early death; whereas, nephropathy and retinopathy progressed slowly and steadily. The study also showed that despite regular follow-up glycemic control progressively deteriorated.
Support: Diabetic Association of Bangladesh

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Risk factors for diabetic retinopathy in Asian Indians.
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Background and Aims: Very few population based studies have looked at the risk factors for diabetic retinopathy in Asian Indians, a high-risk group for diabetes. The aim of the present study is to examine the risk factors for diabetic retinopathy in type 2 diabetic subjects in urban India.
Materials and Methods: Chennai Urban Rural Epidemiology Study (CURES) is a population-based study conducted on a representative population of Chennai (formerly Madras) city in south India. Individuals ^{3} 20 years (n = 26,001) were screened for diabetes. Of the 1529 known diabetic subjects, 1382 (90.4%) participated in the study. 354 newly detected diabetic subjects diagnosed by Oral Glucose Tolerance Test (OGTT) also consented for the study. All the subjects underwent four-field stereo color photography and retinopathy was assessed by Early Treatment Diabetic Retinopathy Study (ETDRS) grading of the color fundus photographs.
Results: The overall prevalence of diabetic retinopathy in the population was 17.6%. The prevalence of diabetic retinopathy was significantly higher in men [164/769: 21.3%] compared to women [138/946: 14.6%], $p<0.0001$. The prevalence of diabetic retinopathy was higher among subjects with proteinuria [29.2% vs 16.8%, $p=0.002$], compared to subjects without proteinuria. There was a linear trend in prevalence of diabetic retinopathy with increase in tertiles of HbA_{1c} [trend chi square: 71.3, $p<0.001$], duration of diabetes [trend chi square: 11.5, $p<0.001$] and triglycerides [trend chi square: 6.31, $p=0.012$]. There was no significant difference in the prevalence of diabetic retinopathy between smokers and non-smokers and subjects with and without hypertension. Logistic regression analysis revealed that male gender [$p=0.012$], duration of diabetes [$p<0.001$], triglycerides [$p=0.019$], HbA_{1c} [$p<0.001$] and proteinuria [$p=0.046$] to be associated with diabetic retinopathy.
Conclusion: Male gender, HbA_{1c}, duration of diabetes, triglycerides and proteinuria are associated with diabetic retinopathy in Asian Indians.

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Cystic fibrosis: prevalence of microvascular diabetic complications
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Background: Cystic fibrosis related diabetes has traditionally been regarded as a mild form of diabetes with a low risk of severe late diabetic complications. Diabetes is rare before 10 years of age. In the second decade, the prevalence of cystic fibrosis related diabetes increases with an annual age-dependent incidence rate of 5%, resulting in a 50% prevalence of diabetes at 30 years of age.
Aim: To investigate whether microvascular complications in cystic fibrosis-related diabetes appear with a clinically relevant frequency, specifically the risk of blindness and renal failure, in a well defined population.
Methods: All patients above ten years of age in our tertiary referral centre for cystic fibrosis are annually carefully screened for diabetes and insulin treatment initiated when appropriate. All patients above 18 years of age with CFRD (n=40) in the Dept. of Endocrinology, Rigshospitalet, were screened for late diabetic complications. Thirty-eight (95%) patients participated in the study. Because of chronic pulmonary infections, all patients were regularly treated with aminoglycoside and 9 lung transplanted patients were treated with cyclosporin. Both drugs are nefrotoxic.
Results: The median age was 32 years (18–55 years). BMI was normal, 20.8 (14.7–32.9). The patients had a relatively long duration of diabetes (12 years (0–31 years)). HbA_1c was 6,95% (5,4–11,2%). Nine patients (27%) had retinopathy, two of which had proliferative retinopathy and needed laser treatement. These patients currently had HbA_1c's of 6,4% and 8,3%, but had HbA_1c's of 11,6% and 12,3%, respectively, 5 years previously. Five patients (16%) had microalbuminuria (30–300 mg/24 h), none had macroalbuminuria. Eight patients (21%) had elevated serum creatinine (>110 µmol/l), one needed dialysis. Seven of these patients were lung transplanted. The GFR of the lung transplanted patients were 35 ml/min/1.73 m^2 (20–73 ml/min/1.73 m^2). GFR was 101 ml/min/1.73 m^2 (64–152 ml/min/1.73 m^2), n=23, in non-transplanted patients. Ten patients (27%) had hypertension (>140/90 mmHg). Six of these were lung transplanted, three of the others had microalbuminuria. Three patients (8%) had decreased sense of vibration. None had claudicatio, MI, TCI or ulcerations of the feet. Serum cholesterol was 4 mM (2.3–6.1 mM).
Conclusion: Patients with cystic fibrosis related diabetes developed diabetic retinopathy and microalbuminuria with a clinically relevant frequency. Severely reduced renal function was frequent, but presumably secondary to treatment with aminoglycoside and cyclosporin.

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Functional MRI activation in type 1 diabetes patients with and without diabetic retinopathy

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Background and Aims: Chronic hyperglycaemia is now emerging as a potential determinant of impaired brain function in type 1 diabetes. Hyperglycaemia may lead to accumulation of potentially toxic glucose metabolites, oxidative stress, accelerated formation of advanced glycation end products and microvascular changes in the brain, analogous to peripheral complications of diabetes. We tested the hypothesis that the presence of retinopathy, as a marker of microvascular disease, adversely affects the regional vasodilatory responses in the brain.
Materials and Methods: We tested 10 type 1 diabetes patients with proliferative diabetic retinopathy (DRP) (mean age: 39.0 ± 2.1 years), and 14 age and gender matched type 1 diabetes patients without DRP (mean age: 40.1 ± 4.1 years). We compared the changes in the blood oxygen level dependent (BOLD) responses during a cognitive task (N-letter back task) following a sequence of euglycaemia (blood glucose 4–6 mM) and acute hypoglycaemia (blood glucose 2.5 mM for 10 minutes), as a mode to test brain response, using functional magnetic resonance imaging (fMRI).
Results: Performance of the N-letter back task declined ($F_{1.7,37.1} = 44.1$ and $P < 0.001$) and reaction time increased ($F_{2.1,45.1} = 26.8$ and $P < 0.001$) if task load increased. Furthermore, performance declined ($F_{1,22} = 5.2$ and $P = 0.03$) and reaction time increased ($F_{1,21} = 6.9$ and $P = 0.02$) if patients became hypoglycaemic, but these deteriorations were not related to task level and patient group. No interaction effect of task level and glycaemic condition and patient group can be seen on performance ($F_{2.2,49.2} = 2.6$ and $P > 0.1$) and reaction time ($F_{3,63} = 0.31$ and $P > 0.8$). Main effects (independent of group) during euglycaemia and hypoglycaemia involved activation in bilateral parietal areas, bilateral frontal areas, bilateral temporal areas, bilateral thalamus and bilateral cerebellum, but also deactivation (which is commonly observed) in the insula, anterior cingulate gyrus, posterior cingulate gyrus, bilateral parietal areas and bilateral frontal areas during euglycaemia. Only the anterior cingulate gyrus and orbital frontal cortex showed an interaction effect of diabetic group and condition: these regions showed less deactivation during hypoglycaemia in the DRP group ($P < 0.05$).
Conclusion: Since task performance did not show this interaction, we conclude that microvascular damage in the brain of patients with retinopathy caused this increased brain response in order to compensate functional loss.
This project was supported by a grant from the Dutch Diabetes Research Foundation (2001.11.012).

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Red dots near the centre of the fovea on digital retinal photographs in diabetic retinopathy screening - 6 month or 12 month review?

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Background and aims: In retinopathy screening, digital retinal photography detects many patients with red dots within one disc diameter (1DD) of the centre of the fovea but little else. Guidelines vary regarding what should be done with these patients. A single case was observed however in which such a patient progressed over 7 months to well established exudative maculopathy with clinically significant macula oedema (CSMO) requiring laser treatment. In the wake of this case we aimed to undertake an audit to gauge how common such cases were.
Material and methods: We audited 135 consecutive patients who when attending for eye screening were found to have red dots within 1DD of the centre of the fovea but little else. They were 78 male and 57 female, median age 58 years (30 - 86). The red dots were in one eye in 111/135 (82%) and both eyes in 24/135 (18%). All were offered 6 month re-screening.
Results: 104/135 (77%) attended for rescreening at 6 months. In 35/104 (34%) the red dots had resolved; in 63/104 (61%) they were still present in one or both eyes. In 6/104 (6%) the retinopathy had progressed such that referral to an ophthalmologist was indicated according to standard European referral criteria - in 5/6 there were hard exudates within 1DD of the centre of the fovea and/or circinate or group of exudates within the macula; in 1/6 there were three large blot haemorrhages near the centre of the fovea. However at ophthalmological review 2-4 months later none of these 6 patients had CSMO and none required laser therapy.
Conclusion: There is a 5% chance of patients with red dots near the centre of the fovea developing macula hard exudates in 6 months; however most do not develop CSMO during this time span. Although 6 month review of such patients would be ideal, because of the resource implications to screening services attempting to reach whole populations, 12 month review may be more pragmatic.

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Volumetric measurements obtained by optical coherence tomography may provide a useful clinical tool for the early detection of macular changes in diabetes

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Background and Aims: Our aim was to investigate the role of optical coherence tomography (OCT) in the early detection of macular changes in patients with diabetes and no apparent visual loss.
Patients and Methods: We performed OCT in 189 eyes of 118 patients with diabetes without any apparent visual loss and 38 eyes of 25 healthy volunteers (control group). All patients underwent a complete ophthalmologic evaluation. The diagnosis of any stage of diabetic retinopathy (DRP) was recorded. Six automated radial OCT scans (OCT Model 2000) were obtained in all eyes with a length of 6 mm centered on the foveola. Central foveal thickness (FT) and macular volume (MV) were assessed, and retinal thickness data were compared with the normative database of the software. Diffuse macular edema (DME) was diagnosed if retinal thickness exceeded the limits of the normative database. Four groups were formed: Control (n=38), DM (diabetes, normal thickness, no retinopathy, n=140), DME (diabetes, DME, no retinopathy, n=17) and DME DRP (diabetes, DME, retinopathy, n=32). Differences between the groups were compared using the Kruskal-Wallis test one-way analysis of variance. In case of significant result Newman-Keuls post hoc analysis was applied.
Results: In the DME DRP group the FT values were markedly higher than in all other groups (C: 168.5 [155–183], DM: 174.5 [159–193], DME: 182 [164–221] and DME DRP 245 [213–284] μm median [interquartile range], $p<0.001$). In the case of MV both groups with DME had significantly higher values than the other groups (C: 6.99 [6.84–7.23], DM: 6.88 [6,61–7.14], DME: 7.53 [7.31–7.66], $p<0.001$, DME DRP: 8.05 [7.52–8.86] mm^3 median [interquartile range], $p<0.001$), and there was significant difference also between these two groups ($p<0.05$).
Conclusion: The increase in macular volume seems to precede the increase in central foveal thickness in patients with diabetes. A possible reason for this could be that the foveola is not necessarily involved in the first pathophysiological changes of the retina in diabetes. Our results implicate that regular OCT-control of all patients with diabetes even in the lack of ophthalmoscopically recognizable alterations may help the early detection of retinal changes.
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The corneal endothelium and thickness in adolescents with type 1 diabetes mellitus

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Background and Aims: To evaluate the systemic factors that contribute to the damage of endothelial cells in diabetic patients, and to compare the endothelial structure and thickness of the cornea in diabetic and nondiabetic patients.
Materials and Methods: The endothelial cell density (ECD) and central corneal thickness (CCT) were investigated in 102 eyes of 102 type I diabetic patients and in 100 nondiabetic patients (100 eyes). The mean diabetic

patients age was 15,31 ± 3,18 years. The mean age in control group was 14,3 ± 2,2 years. Statistical analysis was performed to assess systemic factors (patient age, sex, duration of diabetes mellitus, hemoglobin A1c value, creatine value, diabetes control, microalbuminuria) related to ECD and CCT. The corneal endothelium and thickness was imaged by non-contact microscope Topcon SP-2000P.
Results: SAS STAT (Release 8.2) program, the independent t-test, Kolmogorow-Smirnow test and Bartlett test were used to compare differences between the diabetic and control group. In our study the mean ECD in diabetic eyes was 2470,17 ± 440,22 and was significantly decreased compared to control group (2995,818 ± 266,7). The mean CCT in diabetic eyes was 0,54 ± 0,03 and was significantly increased compared to control group (0,525 ± 0,037). Patient age, duration of diabetes mellitus and creatine value were significantly correlated with ECD. None of systemic factors was correlated with CCT.
Conclusion: Our findings indicate that diabetes mellitus affect corneal endothelium and thickness of cornea in adolescents. Evaluation of endothelium in specular microscope should be performed in all diabetic patients.

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Visual outcome of laser treatment for maculopathy in people with diabetes. Analysis of medical factors associated with diabetic maculopathy in laser treated patients; long term follow up
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Aim: To assess visual outcome of laser treatment for diabetic maculopathy and analysis of associated medical factors.
Background: Diabetic Retinopathy is the leading cause of blindness throughout the world. Maculopathy is prevalent in type 2 diabetes mellitus. Here, we determine the effect of early laser treatment for diabetic maculopathy. The study also determines the prevalence of other medical factors within this cohort.
Materials and Methods: Retrospective study of focal laser treatment for diabetic maculopathy in 92 eyes was undertaken. Follow-up after laser treatment ranged from 3 to 5 years.
Visual acuity was noted at pre-laser and 1 year, 3 years, 5 years following laser treatment.
Type and duration of diabetes, blood pressure control, proteinuria, cholesterol, smoking, presence of vascular disease and HBA1c were analysed. Anti-diabetic treatment and blood pressure treatment were also recorded.
Results: The age of patients ranged from 27 to 87 years with a mean age of 62 years. 90% of the cohort had Type 2 diabetes, with 10% having Type 1 diabetes. Duration of diabetes at initial laser treatment varied from 6 months to 32 years with a mean of 13 years. Blood pressure was above target in 70% of patients prior to laser and continued to be uncontrolled in 85% of the above group, 3 years post laser treatment. This is despite the increase in the number of medications received for hypertension. At 3 years following laser treatment, 85% of patients were noted to have a stable or improved visual acuity. 6.6% were noted to have a decline in visual acuity as a result of maculopathy.
Conclusions: Focal laser treatment is an effective intervention for maintaining/improving visual acuity in patients with diabetic maculopathy.
This study also illustrates that there is high prevalence of uncontrolled hypertension, hypercholesterolaemia and poor diabetic control within this cohort, perhaps reflecting typical type 2 diabetic populations. Perhaps in future a more aggressive treatment approach is necessary, including the need for joint clinics.

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Factors contributing to loss of vision in patients registered blind and partially sighted secondary to diabetic retinopathy
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Background and Aims: Despite a programme of regular retinal screening and treatment for diabetes and hypertension, some patients still lose their vision from diabetic retinopathy. In order to improve our outcomes, we aimed to determine the incidence of visual loss and any factors associated with this in our patients.
Materials and Methods: We conducted retrospective review of those patients registered blind or partially sighted secondary to diabetic retinopathy in Leeds from 2002–2004. We examined the cause of visual loss, any laser treatment, the adequacy of laser treatment and non-attendance rates. The clinical records were studied for information on the type and known duration of diabetes, treatment by primary or secondary care, time from referral to assessment in the ophthalmology clinic and the subsequent time to loss of sight registration. The blood pressure, HbA_{1c} and biochemistry result at referral and registration were noted.
Results: 24 patients, 11 men and 13 women, mean age 54 (24–89) years were registered either blind (8) or partially sighted (16) over a 40 month period. This equates to 7.2 annual registrations. The population of Leeds is 768,061 and the known diabetic population is approximately 21,500 (2.8%). The registration rate for blindness or partial sight in the diabetic population was 0.033% per year. Eight patients were treated in primary care and sixteen in secondary care for diabetes.Five patients received annual retinal screening and nineteen had not. The mean HbA_{1c} at referral to ophthalmology was 8.6% (7.4 to 10.4) and at registration 7.3% (6.9 to 10.5). The mean creatinine level at referral was 76 umol/L (75–88) and 107 umol/L (90–110) at registration. The BP was 150/ 84 (110/60–190/100) and 104/83 (100/60–140/90), respectively. At registration, the known diabetes duration was 14–25 years. Thirty percent of patients were considered as non compliant (>/= 1 missed ophthalmic appointments).Only 5 patients were on retinal screening programme. The mean time from referral to ophthalmological assessment was 58 (1–133) days.Thirty three percent of patients were assessed within one month and sixty seven percent within two months.The mean interval from initial assessment to registration was 4.8 years.The causes of visual loss were proliferative retinopathy in 60% and maculopathy in 40% of cases. Forty seven percent of patients presented late as they already had symptoms of visual loss. In 2 patients laser treatment was given later than 13 weeks after assessment. There was a failure of adequate response to laser treatment in 87% of those who had it.
Conclusion: The annual rate of registration for blindness or partial sight in Leeds is low.Contributory factors to visual loss included some cases of late presentation, some treatment failure and 19 who had escaped annual retinal screening.The biochemistry and blood pressure values were not particularly bad in these cases.

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Automated detection of diabetic retinopathy in digital retinal images: high sensitivity and specificity but a cautionary tale
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Background and Aims: Diabetic retinopathy (DR) is the commonest cause of blindness in the working age population of the developed world. Digital retinal photography provides an opportunity for the establishment of DR screening programs. A national program is being rolled out in the United Kingdom. Employment of trained graders to assess retinal images is a significant cost component of the screening process. Automated detection of DR in digital images is an attractive proposition; it could potentially reduce staffing costs, it could improve reliability, and it could be particularly useful as part of a systematic audit and quality control system. The aim of this study was to develop and evaluate a system for the automated classification of diabetic retinal images. Previous work on low resolution images provided encouraging results; the present study included conversion to the use of higher resolution images and the use of macular and nasal fields.
Materials and Methods: Digital retinal images of 248 consecutive patients from a DR screening program were used. Images comprised 45 degree macula-centred and nasal fields from each eye at a resolution of 1024 × 1024 pixels in RGB colour TIFF format. Image analysis comprised: pre-processing; identification of optic disc and blood vessels; image segmentation and classification of candidate regions into dark lesions, bright lesions or noise. Artificial neural networks were used for lesion classification. Images from the first 140 patients were used for development, training and preliminary evaluation of the system. Sensitivity and specificity of DR detection was calculated by comparison with reference standard grading by an experienced clinician blinded to the results of the computer analysis. System performance was assessed at several different settings and any sight threatening DR missed by the system was noted. Optimum system settings, giving good sensitivity and specificity without missing any sight threatening disease, were determined.
After this, an independent evaluation was performed using images from the remaining 108 patients. This independent evaluation „test set" was processed once only, at the pre-determined optimum settings, to simulate clinical use of the system.
Results: On the training data, a wide range of results for sensitivity and specificity could be obtained, depending on system settings. Setting judged as optimum gave sensitivity for detection of any DR as 95%, with specificity

74%. At these settings no sight-threatening DR was missed. Running the system once only, at the pre-determined settings, on the independent test set, gave sensitivity for detection of any DR as 83%, with specificity 51%. At these settings one patient with potentially sight-threatening maculopathy was missed by the system.
Conclusion: High sensitivity and specificity for automated detection of DR can be achieved, and a clinically usable system may eventually be possible. However, the results from the second part of our study highlight the importance of evaluation on an independent test set, and the need to interpret preliminary sensitivity and specificity results with caution. In view of the high degree of variability in retinal images there is a need for much more work before a clinically reliable automated DR detection system could be deployed.
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Fast effect of low dose octreotide on fluorescein leakage in early diabetic retinopathy. Preliminary report
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Background and Aims: Growth Hormone has been implicated in the pathogenesis of diabetic retinopathy and hypophysectomy has been tried as treatment of proliferative retinopathy. The present study investigates the effect of inhibition of Growth Hormone secretion by a somatostatin analog, octreotide, on earlier abnormalities of diabetic retinopathy, namely Fluorescein leakage from the retinal vessels.
Materials and Methods: We studied seven patients with type 1 diabetes, 4 male and 3 female, of age 25.2 years (22-35), diabetes duration 9.7 years (5–13), BMI 24.2 kg/m^2 (22.2–25.1) and daily insulin dose 43.8 (35–52) units, who at Fluorescein Angiography, had Fluorescein leakage on the macular region but not proliferative retinopathy. All patients were treated with 45 μg octreotide daily (Somatostatin analogue) given subcutaneously in three doses for 4 weeks, when a second Fluorescein Angiography was done. We used four pairs of photographs for each patient from the first and second Fluorescein Angiography, matched for timing and retinal area, which were compared by two experienced ophthalmologists, who did not know either the chronological order of the photographs or the patients. Differences between the two observers in the evaluation of the 28 pairs of photographs occurred only in two occasions. The final evaluation of these two pairs was done by a third observer. As "improvement" was considered the decrease of Fluorescein leakage in all 4 pairs of photographs of each individual patient.
Results: After 4 weeks of treatment with octreotide, mean daily blood glucose (self-monitoring, 3 times/day) improved, 168.1 ± 12.2 vs 138.2 ± 15.2 mg% and HbA_{1c} decreased, 9.2 ± .5 vs 7.2 ± .3%, p<.01 for both. Two patients developed mild diarrhea, not needing special treatment or cessation of the octreotide. Four from the seven patients (57%) showed clear-cut improvement of Fluorescein leakage, two patients showed no improvement and one patient (14%) showed deterioration. The improvement of blood glucose control with octreotide is expected and theoretically could contribute to the improvement of Fluorescein leakage. However the effect of glycaemic control on diabetic complications is known to be a very long procedure, a fact indicating octreotide treatment as the most probable cause of improvement of the diabetic retinopathy.
Conclusion: This pilot study provides evidence that inhibition of Growth Hormone secretion by low dose octreotide, for a relatively short period, improves early stages of diabetic retinopathy (Fluorescein leakage), underlining the role of growth factors early in the development of diabetic retinopathy and suggesting there is possibility for future therapeutic intervention.

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Pancreas transplant alone (PTA) has beneficial effects on diabetic retinopathy
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Background and Aims: Pancreas transplant alone (PTA) can significantly improve the quality of life of Type 1 diabetic patients, and can also eliminate diabetes acute complications, such as hypoglycemic and/or hyperglycemic episodes. However, the effects of PTA on long-term complications of diabetes, including retinopathy (DR), are still unsettled. We evaluated whether restoration of long-lasting normoglycemia by PTA might have beneficial effects on DR.
Materials and Methods: We studied the course of DR in 34 patients (age: 37 ± 8.8 years; males/females: 18/16; BMI: 23.7 ± 3 Kg/m^2; duration of diabetes: 23 ± 11 years) bearing a successful PTA. Follow-up was 21 ± 7 months (range: 12 to 36 months). Patients were examined with corrected visual acuity, slit lamp examination, measurement of intraocular pressure, indirect and direct retinoscopy, two non-stereoscopic 45° retinal photographs, and retinal angiography. DR was classified according to the Eurodiab Study as non-proliferative retinopathy (NPDR) or laser-treated and/or proliferative retinopathy (LT/PDR). Data were compared with those of 37 matched Type 1 diabetic patients, treated with intensive insulin therapy.
Results: At the last follow-up visit, PTA patients were normoglycemic (fasting plasma glucose: 88 ± 11 mg/dl; HbA1c: 5.3 ± 0.4%, C-peptide: 2.9 ± 1.5 ng/ml), without exogenous insulin administration. Visual acuity was 0.86 ± 0.2 and 0.78 ± 3 lines before PTA and at the last control, respectively (NS). After PTA, 1 patient required cataract extraction. Ocular tone did not differ between pre- (14 ± 2.0 mmHg) and post-transplant (15 ± 2 mmHg) examinations. Before transplantation, 12 patients (35%) had NPDR, and 22 patients (65%) had LT/PDR. In the NPDR group, 4 (33%) patients improved of 1 lesion grading, and 8 (67%) patients showed no change of retinal status. In the LT/PDR group, stabilization was observed in 17 (77%) patients, and worsening in 5 (23%) patients. The number of improved/stabilized patients was significantly (p<0.01) higher in the transplanted (29, 85%) than in the control group (16, 43%).
Conclusion:
Successful PTA, by restoring sustained normoglycemia, can positively affect the course of diabetic retinopathy.

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Hypertension: clinical aspects

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Hemoglobin A_{1c} is associated with resting and exercise blood pressures

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Background and Aims: The risk for cardiovascular disease and total mortality associated with HbA1c concentrations increases continuously in adults without diabetes. In healthy non-diabetic and non-hypertensive men, fasting blood glucose is independently associated with resting and exercise blood pressures and development of elevated blood pressure. Aim of the study was to investigate the relationship between blood pressure response to exercise test and indices of glucose metabolism in healthy adults.
Materials and Methods: We studied 95 healthy subjects (44 ± 13 y) without established coronary heart disease who underwent routine clinical cycle ergometer test. Measurements included body weight, blood pressure, fasting plasma glucose (FPG) and insulin (FPI), HbA_{1c}. Insulin resistance was estimated by homeostasis model assessment (HOMA IR).
Results: BMI was 25 ± 4 kg/m^2, mean blood pressure 92 ± 11 mmHg, FPG 5.0 ± 0.5 mmol/l, FPI 8.9 ± 4.1 μU/ml, and HbA_{1c} 5.3 ± 0.4%. Resting systolic blood pressure showed a positive association with age ($p<0.0001$), BMI ($p<0.0001$), FPG ($p<0.001$), and HOMA IR ($p<0.0001$). Maximal systolic blood pressure had a highly significant positive association with resting systolic blood pressure ($p<0.0001$). Moreover, it was positively associated with BMI ($p<0.001$), FPG ($p<0.001$), and HOMA IR ($p<0.0001$). Exercise-induced increase in systolic blood pressure was correlated positively with age ($p<0.01$), resting systolic and diastolic blood pressures ($p<0.05$ and 0.01, respectively), and HbA_{1c} ($p<0.001$).
Stepwise multiple regression analyses found diastolic blood pressure (coefficient 0.005, F value 7) and HbA1c (0.16, 11) to be significantly associated with an exaggerated blood pressure response to exercise testing ($|r| = 0.44$, $p<0.0001$).
Conclusion: Present unexpected finding is that HbA_{1c} levels markedly influenced the exercise-induced increase in systolic blood pressure in healthy individuals. This is the first report which links a measure of long-term glycemic control to variations in systolic blood pressure during exercise in healthy people. Since the HbA_{1c} test reflects mean glycemia over the preceding 2-3 months, measurement permits to assess also postprandial glucose values that have been associated with increased cardiovascular risk independent of fasting plasma glucose in some epidemiological studies. Thus, glycosylation phenomenon might be relevant also in healthy subjects, as suggested by the observed association of HbA_{1c} with cardiovascular mortality in adults. Indeed, alterations in matrix proteins from advanced glycation end-product affect artery compliance.

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Olmesartan vs. diltiazem in type 2 diabetic patients with hypertension and microalbuminuria. Effects on blood pressure and albumin excretion

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Background and Aims: To study the effect of Olmesartan vs. Diltiazem on blood pressure and albumin excretion in type 2 diabetic patients with hypertension and persistent microalbuminuria, in a randomised, open study.
Materials and Methods: From our Diabetes Clinic, 60 patients with type 2 diabetes mellitus, essential hypertension (naive, or otherwise after 1 month washout), persistent microalbuminuria and normal renal function were randomly assigned (2/1) to Olmesartan 40 mg (40 patients) or Diltiazem 300 mg (20 patients) daily for 24 weeks. Height, weight, waist perimeter, blood pressure, heart rate, fasting plasma glucose, glycated hemoglobin, creatinine, sodium, potassium, total cholesterol, triglycerides and urinary albumin excretion were measured by standard clinical procedures, before and after treatment; tolerance was assessed after treatment.
Results: The mean age was 60.3 ± 11.1 years; 52.3% were women. SBP decreased from 153.4 ± 11.7 to 135.6 ± 11.9 mmHg ($p < 0.001$, paired t-test) with Olmesartan and from 151.0 ± 11.5 to 133.6 ± 11.7 ($p < 0.001$) with Diltiazem; DBP decreased from 93.4 ± 9.7 to 81.6 ± 9.9 ($p < 0.001$) with Olmesartan and from 92.0 ± 10.6 to 80.3 ± 9.7 ($p < 0.001$) with Diltiazem. Albumin excretion decreased from 133.7 ± 67.8 to 83.5 ± 68.2 μg/min with Olmesartan ($p < 0.001$) and from 143.6 ± 76.5 to 119.7 ± 59.4 with Diltiazem ($p = 0.012$). The differences between treatments were significant for albumin excretion ($p = 0.021$, non-paired t-test) but not for SBP and DBP. The rest of the studied variables remained unchanged. There were no serious side effects or treatment withdrawals.
Conclusion: Both Olmesartan 40 mg and Diltiazem 300 mg were safe and effective in the treatment of type 2 diabetic patients with hypertension and persistent microalbuminuria; however, Olmesartan reduced albumin excretion more effectively than Diltiazem.

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Arterial pulse pressure increases according to diabetes duration, independently of age in patients with type 1 diabetes

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Background and Aims: Arterial pulse pressure (PP) reflects arterial stiffness and is considered as an independent cardiovascular risk factor. However, studies regarding PP in type 1 diabetic patients are scarce. The aim of the study was to investigate the influence of the duration of type 1 diabetes on PP, as well as to correlate PP with the quality of blood glucose control and the presence of microalbuminuria (μA) and/or drugs blocking the renin-angiotensin system.
Material and Methods: A total of 159 type 1 diabetic patients (20–60 years) were evaluated with a continuous noninvasive arterial blood pressure monitoring (Finapres®). Recordings were performed in standing position (1 min), in squatting position (1 min), and again in standing position (1 min). Presented data correspond to average PP (systolic – diastolic arterial pressure) values calculated during the overall recording in the 3 positions. Blood glucose control was assessed by concomitant HbA_{1c} levels. Thirty-one patients were treated with an ACE inhibitor or an angiotensin AT1 blocker (ARB) because of μA antecedent. Subjects were retrospectively separated according to: 1) diabetes duration: group 1: < 10 years (n = 39); group 2: 11–20 years (n = 45); group 3: 21-30 years (n = 57); and group 4: >30 years (n = 18); 2) concomitant HbA1c levels (< or ≥ 8%); 3) μA (< 30 or ≥ 30 mg/l); and 4) current treatment with ACE inhibitor or ARB (yes or no). In order to differentiate the effects of duration of diabetes from the effect of increasing age, healthy subjects were used as controls and matched for age, sex and body mass index (n = 30 in each subgroup).
Results: PP was higher in men than in women, in both diabetic (58 ± 15 vs 50 ± 14 mm Hg; $p < 0.001$) and non-diabetic (55 ± 14 vs 47 ± 12 mm Hg; $p < 0.001$) subjects. PP increased progressively throughout the 4 subgroups according to diabetes duration (47 ± 16 vs 51 ± 13 vs 59 ± 14 vs 62 ± 12 mm Hg, respectively). There was a marked difference between groups 1–2 and groups 3–4 (49 ± 14 vs 59 ± 14 mm Hg; $p < 0.00002$). Such a progressive PP increase was not observed in non-diabetic subjects in the same age range (mean of 35 years for groups 1–2 vs 46 years for groups 3–4) : 51 ± 12 vs 50 ± 15 mm Hg; NS. Percentage of subjects with PP ≥ 60 mm Hg was similar in diabetic and non-diabetic individuals in groups 1–2 (27 vs 25%, NS); however, in groups 3–4, the prevalence of subjects with PP ≥ 60 mm Hg was significantly higher in diabetic patients than in controls (44 vs 27%, $p < 0.05$). The difference in PP between the four groups of diabetic patients, already present in standing position, was amplified in squatting position: the coefficient of correlation between individual PP values and diabetes duration data increased from $r = 0.289$ ($p < 0.01$) while standing to $r = 0.362$ ($p < 0.001$) in squatting position. PP was similar in patients with HbA_{1c} <8% (54 ± 14 mm Hg) or ≥ 8% (55 ± 16 mm Hg). No significant differences were observed in patients with μA (57 ± 17 mm Hg) vs without μA (54 ± 14 mm Hg) and in patients receiving (56 ± 14 mm Hg) or not receiving (54 ± 15 mm Hg) an ACE inhibitor or an ARB.
Conclusion: Type 1 diabetes was associated with a progressive increase in PP according to the duration of the disease, in an age range where no significant influence of age was observed in a non-diabetic population. PP was not significantly influenced by concomitant HbA_{1c} levels, and no variation in PP was associated with μA or blockade of the renin-angiotensin system.

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Importance of blood pressure control in the longterm treatment of diabetes: results of a 10 year simulation with the EAGLE model

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Background and Aims: Based on published data from a German patient cohort, the development of macrovascular complications was assessed in type 2 diabetes patients using the EAGLE diabetes simulation model. The influence of a tight blood pressure control in addition to glycemic control was evaluated over a 10 year period.
Materials and Methods: The EAGLE (Economic Assessment of Glycemic Control and Longterm Effects) model is a stand-alone computer simulation program providing micro-simulations of virtual patient cohorts over n years in 1-year cycles. Outcomes are calculated over time as cumulative incidence including micro- and macrovascular complications and death. Subsequent health economic calculations are constructed from the simulation results. The model algorithms are based on published trial results from DCCT, UKPDS, and WESDR. Main influence factors driving the development of complications are HbA1c, blood pressure, lipid values, age, duration of diabetes and treatment regimen. Simulation baseline values were derived from the type 2 cohort of the German TEMPO® study: age 64 ± 11 years, diabetes duration 10 ± 8 years, 49% male. Mean HbA1c drops from 7,7 ± 1,8% to 7,2 ± 1,4% within the first simulation year with a subsequent increase of 0.2% per year. Macrovascular outocomes with a hypertension prevalence of 80% in the base case were compared to outcomes with 50% of hypertensive patients receiving intensified or less tight blood pressure control.
Results: After 10 years the mean HbA1c in the simulated cohorts was 9% respectively. Mean systolic blood pressure in the base case was 154 mmHg compared to 139 mmHg in the less tight and 131 mmHg in the intensive contolled cohort. The overall risk for development of macrovascular events droped by 17% in the tight controlled group and by 11% in the less tight controled goup compared to the base case. The risk reductions for non-fatal myocardial infarction were 11% and 8%, for heart failure 13 and 9% and for stroke 33 and 23%. Concomitantly the overall mortality rates were reduced by 13% and 8% respectively.
Conclusion: The simulations demonstrate that with equal glycemic control blood pressure control has an imporatnt impact on the longterm outcomes in a diabetes cohort. Even with a less tight control macrovascular events and mortality can be reduced substantially.

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Optimising blood pressure control in the type 2 population is more achievable in the absence of microalbuminuria or nephropathy

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Background and Aims: Optimising blood pressure control in type 2 diabetes mellitus demands effective interventions to achieve recommended targets of 140/80 mmHg or less. For type 2 patients with microalbuminuria or nephropathy there is considerable benefit gained by optimising BP to below 130/80 mmHg, which may be difficult to achieve. We report a comparison between 2 groups of type 2 patients attending protocol driven nurse-led clinics to optimise BP control.
Materials and Methods: Patients were referred from routine consultant outpatient clinics and enrolled into either 1: a nurse-led clinic to optimise BP control or 2: if microalbuminuria or nephropathy was present a nurse-led clinic to instigate angiotensin receptor blockade (ARB) therapy and optimise BP control.
Group 1: "Non-renal" 108 patients with hypertension, BP >140/80 mmHg and taking at least one anti-hypertensive drug. The primary aim was to optimise BP levels to 140/80 mmHg or less.
Group 2: "Renal" 81 patients with microalbuminuria or nephropathy, 70 (86%) also had BP above 130/80 mmHg. The primary aim was to optimise BP to 130/80 mmHg or less and to instigate treatment with an ARB (irbesartan to maximum dose of 300 mgs daily).
Both clinics used a therapeutic management algorithm to guide the nurse on adjustments and optimisation of antihypertensive therapy on a monthly review basis until target BP was achieved.
Results: There were fewer males in group 1, 24 (42%) v 45 (67%), (p = 0.001), but no significant difference in age, duration of diabetes and HbA1c. Group 1 had higher systolic BP, (181 ± 20 v 151 ± 22, p <0.001) and diastolic BP, (86 ± 12 v 76 ± 14, p <0.001) at referral. On completion, target BP was achieved in 94 (87%) group 1, compared to 60 (74%) group 2, (p =0.04). Group 1 patients also required less intervention to achieve target BP, requiring fewer visits, (4 ± 2, v 6 ± 3, p = 0.009), and a smaller number of patients in group 1 required further intervention from a physician during attendance at the nurse clinic, (4% v 21%, p <0.001). The attendance rate was also better in group 1 patients, (95% v 84%, p <0.001).
Conclusion: Nurse-led clinics can be used effectively to optimise BP in the type 2 population. However, patients with microalbuminuria or nephropathy require more intensive intervention and more input from a physician. The attendance rate is generally good for these clinics, although non-attendees become more likely as intervention becomes more prolonged and complex.

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Refractory hypertension in type 2 diabetes

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Background and Aims: Current European and US guidelines (European Society of Hypertension, JNC 7), suggest a BP target of < 130/80 mm Hg for patients with type 2 diabetes; this may be difficult to achieve in clinical practice. The prevalence of refractory hypertension in a general diabetes clinic is unknown, as are the reasons for failure to achieve current BP targets. The aim of the present study was to define the prevalence and describe the factors associated with refractory hypertension in patients with type 2 diabetes in a secondary care setting.
Materials and Methods: Patients with type 2 diabetes attending a secondary care diabetes clinic over a one year period (1/10/03 to 30/09/04) were identified using a computerised clinical database (Diabeta3). Refractory hypertension was defined as failure to achieve target blood pressure in patients who are adhering to full doses of an appropriate 3-drug regimen that includes a diuretic. Data were analysed by multivariate logistic regression analysis and analysis of variance and expressed as mean +/– SE.
Results: A total of 4310 patients attended the clinic during the above period. 440 patients had a BP < 130/80 mm Hg on no antihypertensive drugs. The remaining 3870 patients (90%) had a BP above the target of 130/80 mm Hg or were receiving antihypertensive treatment. Of those, 698 (18%) achieved a BP <130/80, 1041 (27%) had a suboptimal BP (130–139/80–89 mmHg), 1466 (38%) had mild (140–159/90–99), 474 (12%) had moderate (160–179/100–109) and 191 (5%) had severe (≥180/≥110 mmHg) hypertension. Out of the total clinic population 1159 patients (27%) achieved the currently recommended target. 706 (18%) of all hypertensive patients had refractory hypertension. Age, BMI, serum creatinine and urine albumin to creatinine ratio (ACR), were all significantly higher in those with refractory compared to those with non-refractory hypertension; age 66 +/– 0.4 vs 62 +/– 0.1 years, p<000.1, BMI 32 +/– 0.2 vs 30 +/– 0.1, p<000.1, serum creatinine 110 +/- 2.7 vs 93 +/- 0.9 umol/l, p<000.1 and ACR 7.7 +/– 0.6 vs 5.7 +/– 0.2, p=0.001. In multivariate analysis, age (p<0.001), serum creatinine (p<0.001), ACR (p=0.009) and BMI (p<0.001) were independently associated with refractory hypertension. There was no association between a higher HbA1c, serum total cholesterol, triglycerides (TG) and refractory hypertension (HbA1c 7.7 +/– 0.5 vs 7.8 +/– 0.2%, total cholesterol 4.6 +/– 0.2 vs 4.6 +/– 0.3 mmol/l, TG 1.9 +/– 0.4 vs 2.0 +/– 0.2 mmol/l in the refractory and non-refractory hypertension group respectively). Antihypertensive agents used were: beta-blockers 19%, diuretics 39%, calcium channel blockers 36%, alpha blockers 14%, ACE inhibitors 49%, angiotensin receptor antagonists 18%, other 2.8%.
Conclusion: A significant proportion of patients with type 2 diabetes have refractory hypertension. Worse renal function (lower GFR or greater proteinuria), and greater weight are important determinants of refractory hypertension. Investigation and management strategies for these patients need to be developed.

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Reversible endocrine causes of hypertension in type 2 diabetic patients with poorly controlled blood pressure

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Background and Aims: Aggressive blood pressure (BP) control has been shown to significantly reduce cardiovascular morbidity and mortality amongst diabetic (DM) patients. However, adequate BP control is achieved in only a small proportion of diabetics. Recent evidence suggests that 5–8%

of adult hypertensive patients have underlying primary hyperaldosteronism (PHA).
Materials and Methods: We therefore set out to prospectively evaluate the prevalence of secondary endocrine causes of hypertension, in particular PHA, in 90 patients [36 males; median age 59 yrs (range 27–73 yrs)] with type 2 DM with poorly controlled BP. Median duration of DM and hypertension was 9 (0–36) and 10 yrs (1-40) respectively. Median number of antihypertensives in use was 3 (2-5), majority (35%) being on 3 antihypertensive medications.
Results: Eight patients (8.8%) had a positive screening plasma aldosterone concentration (PAC) to plasma renin activity (PRA) ratio of > 550 (with PAC > 416 pmol/L). One patient unequivocally suppressed the PAC to 69 pmol/L during an intravenous saline loading test (SLT), excluding PHA. Of the remaining 7 patients (7.7%), six failed to suppress their PAC to < 280 pmol/L (10 ng/dL) and one to < 140 pmol/L (5 ng/dL) during intravenous SLT, consistent with the diagnosis of PHA. CT scan of the adrenals detected an unilateral adenoma in 4 and bilateral adrenal hyperplasia (BAH) in the other 3 patients. Adrenal venous sampling (AVS) localized the lesions to the same side as the CT scan in all 4 patients with adenomas. Three of these patients have undergone surgery (the fourth patient is due for surgery in the near future); histology was consistent with an adenoma in 2 and hyperplasia in 1. Of the 3 patients with hyperplasia on CT, AVS failed to show a lateralization in two. However, in one patient, AVS lateralized the lesion to the right side, despite there being no definite adenoma visible on the scan. This patient is undergoing further evaluation prior to considering a right adrenalectomy.
One patient (1%) was confirmed to have ACTH-independent Cushing's syndrome due to a 2.5 cm right adrenal adenoma and is scheduled for surgery soon. She had presented with uncontrolled hypertension, hypokalemia and had mild Cushingoid features. Eight patients (8.8%) had only borderline elevations (< twice the upper limit of normal) in 24-hrs urine free catecholamine and metanephrine levels and were hence not investigated any further.
Conclusion: Our study reveals a high prevalence of PHA in type 2 DM patients with poorly controlled BP (7.7%). We therefore recommend screening for PHA, by using a random PAC to PRA ratio, in all type 2 diabetic patients with poorly controlled BP on 3 antihypertensive medications. Identification and treatment of this reversible form of hypertension will have a significant impact in the management of a subset of diabetics with hypertension. Screening for Cushing's syndrome/phaeochromocytoma should only be undertaken in the presence of other suggestive clinical features of these conditions.
This study is being supported by a grant from the Singapore National Medical Research Council.

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Neuropathy: treatment

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Long-term effect of quinapril or losartan or their combination on diabetic autonomic neuropathy over a period of two years
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Background and Aims: To investigate the effect of angiotensin converting enzyme inhibition or angiotensin receptors blockers or their combination on definite Diabetic Autonomic Neuropathy (DAN). DAN is major contributor to overall morbidity and mortality in patients with diabetes mellitus (DM).
Materials and Methods: Sixty-two patients (34 women, 24 with type 1 DM, aged 51.7 ± 13.9 y, DM duration 17.8 ± 7.3 y) with DAN were studied for a 24 months time-period. No patient with coronary artery disease and arterial hypertension included. Patients were randomly allocated in 3 groups: A (n=20), B (n=22), C (n=20) receiving 20 mg quinapril (Q), 100 mg losartan (L) and 20 mg Q +100 mg L respectively. All patients had 2 or more of the following 4 Cardiovascular Reflex Tests (CRT) abnormal. The R-R variation during deep breathing assessed by Expiration/Inspiration ratio (E/I), Mean Circular Resultant (MCR, vector analysis) and Standard Deviation (SD), Valsalva maneuver (VALS), 30:15 ratio (30:15) and Postural Hypotension (PH) were used. CRT performed every 12 months. Quantitative changes in all parameters were analyzed using ANOVA model.
Results:

	A-baseline	A- 2 years	B-baseline	B- 2 years	C- baseline	C- 2 years
E/I	1.11 ± 0.06	1.23 ± 0.12*	1.08 ± 0.06	1.15 ± 0.09*	1.08 ± 0.04	1.17 ± 0.10*
MCR	18.1 ± 6.2	38.7 ± 20.5*	14.0 ± 9.0	24.9 ± 16.1*	14.6 ± 9.6	28.1 ± 16.0*
SD	31.1 ± 11.9	56.6 ± 23.0*	27.5 ± 14.0	37.9 ± 18.5*	32.2 ± 15.5	49.4 ± 23.8*
VALS	1.48 ± 0.28	1.56 ± 0.33	1.44 ± 0.26	1.44 ± 0.20	1.43 ± 0.28	1.43 ± 0.22
30:15	1.15 ± 0.12	1.18 ± 0.12	1.10 ± 0.10	1.15 ± 0.13	1.09 ± 0.07	1.14 ± 0.11
PH	16.0 ± 11.8	10.4 ± 6.1	14.8 ± 12.7	9.5 ± 8.0	16.1 ± 13.3	12.5 ± 9.6

Significance was accepted for *,p<0.05.

Conclusion:
DAN was improved after two years of treatment with quinapril, losartan, or their combination. Improved autonomic balance may be of clinical importance in long-term prognosis of DM patients.

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Clinical characteristics and pain management in patients with painful diabetic neuropathy (PDN) in general practice settings in the United Kingdom
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Background and Aims: Diabetic neuropathies, a family of nerve disorders caused by diabetes mellitus affect nearly 45% of diabetes patients during the course of their disease. Many patients with diabetic neuropathy experience chronic pain. Our objective in the present study was to describe the demographic and clinical characteristics of persons suffering from painful diabetic neuropathy (PDN) and their use of pain medications.
Materials and Methods: Using the GPRD, we identified 1,647 annonymized patients having at least one medical record with a diagnosis code for PDN between 1998 and 2001. Prevalence of select clinical comorbidities and use of pain medications was examined.
Results: Patients were 64.1 ± 12.6 years old (57.9% were male); over a third (43.9%) had other chronic pain-related comorbidities (arthritis, arthropathies, and musculoskeletal pain were the most common [35.6%]); 43% had chronic non-pain related comorbidities, hypertension (18.0%), coronary heart disease (10.6%) and affective disorders (8.3%) were the most common. A majority (89.9%) received at least one and nearly two third (64.7%) received two or more different types of pain medications; non-steroidal anti-inflammatory analgesics (NSAIDs) were the most common (60.3%). Approximately one third (37.9%) received tricyclic antide-

pressants. Use of other medications with clinical evidence demonstrating efficacy in PDN was less than 1 out of every 5 patients: opioids (16.2%); antiepileptics (18%); carbamazepine (12.1%); and gabapentin (5.8%). Moreover, average daily doses of select medications among patients who received these medications were lower than those recommended for neuropathic pain: amitriptyline (39.4 ± 39.2 mg in PND patients vs. 50 mg–150 mg, recommended dose); carbamazepine (459.3 ± 485.5 mg in PND patients vs. 400–800 mg recommended dose); gabapentin (988.8 ± 748.6 mg in PND patients vs.1800 mg recommended dose).
Conclusion: Results indicated that over a half of PDN patients received NSAIDs, a medication class with no proven efficacy for PDN. Over a third of PDN patients also suffered from other chronic nociceptive pain conditions. Thus, it is conceivable that PDN patients received NSAIDs for their nociceptive pain. However, given the low use of neuropathic pain-related medications, the possibility that at least some PDN patients were receiving NSAIDs for neuropathic pain cannot be ruled out. Further, PDN patients who did receive neuropathic pain-related medications, received lower than recommended daily doses. The frequent use of NSAIDs, combined with the low use and doses of medications with evidence of efficacy in PDN may suggest potentially sub-optimal pain management in these patients.
Support: Pfizer Inc

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Efficacy, safety, and tolerability of pregabalin treatment for diabetic peripheral neuropathy: findings from 6 randomized controlled trials
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Background and Aims: Pregabalin is an alpha-2-delta ligand proven effective for the treatment of neuropathic pain associated with diabetic peripheral neuropathy (DPN), postherpetic neuralgia, and spinal-cord injury, as well as the pain associated with fibromyalgia syndrome. In multiple randomized, double-blind, placebo-controlled trials, pregabalin has been found to consistently reduce the often difficult-to-treat neuropathic pain experienced by DPN patients, resulting in improvement of their overall health status. Using data from a large patient cohort formed by pooling 6 clinical trials, we studied the overall efficacy, safety, and tolerability of pregabalin treatment for neuropathic pain associated with DPN.
Materials and Methods: Data from 6 randomized, double-blind, placebo-controlled studies were pooled and analyzed. 1346 DPN patients were studied: 473 patients received placebo and 873 received pregabalin (176, 266, and 431 patients received 150, 300, and 600 mg/d, respectively). The primary efficacy measure was endpoint mean pain score derived from patient-recorded daily pain diaries (11-point scale: 0 = no pain, 10 = worst possible pain).
Results: Reductions in endpoint mean pain score were significantly larger in patients treated with pregabalin than in those receiving placebo. Observed reductions were -2.04, -2.35, and -2.74 points for patients receiving pregabalin 150, 300, and 600 mg/d, respectively, compared with -1.48 for patients receiving placebo ($p \leq 0.007$). A total of 27%, 39%, and 46% of pts on 150, 300, and 600 mg/day pregabalin, respectively, reported pain reductions ≥50% from baseline to endpoint while only 22% of PBO patients reported comparable reductions. Treatment-emergent adverse events (AEs) were generally mild to moderate. Dizziness and somnolence were the most common AEs (reported by 12% and 5% of pregabalin-treated patients versus 4% and 3% of placebo patients respectively). Pregabalin was well tolerated: AEs led to withdrawal in 11% of pregabalin patients and in 4% of placebo patients.
Conclusion: Pregabalin treatment significantly reduced the pain experienced by patients with painful DPN, with as many as 46% of patients reporting pain reductions ≥50%. Pregabalin was well tolerated and adverse events were generally mild to moderate in intensity.
Support: Pfizer Inc

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Pregabalin significantly improves overall clinical status and health-related QoL in patients with diabetic peripheral neuropathy: findings from 6 randomized controlled trials
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Background and Aims: Painful diabetic peripheral neuropathy (DPN) affects the lives of millions of patients with diabetes. Despite advances in pain management, painful DPN is often treated with medications that either have been shown to be ineffective or are considered effective but are sub-optimally dosed. Pregabalin, a CNS ligand with analgesic, anxiolytic, and anticonvulsant properties, has been proven effective for DPN treatment. We investigated the effect of pregabalin treatment on the perceptions of overall improvement in clinical status and health-related quality of life (HRQoL) reported by patients suffering from painful DPN.
Materials and Methods: Six randomized, double-blind, placebo-controlled trials of 5–12 weeks' duration and comprising 1346 patients were studied. Of the 1346 patients in this analysis, 473 patients received placebo (PBO) and 873 patients received pregabalin (176, 266, and 431 patients on 150, 300, and 600 mg/d, respectively). Patients' perceptions of overall improvement were assessed using "Patient's Global Impression of Change" measurements (1-7 scale) at study end. HRQoL changes from baseline to study termination were determined using the Short Form 36 Health Survey (SF-36; 0–100 scale).
Results: At study end, overall improvement in clinical status was reported by 65%, 74%, and 80% of patients treated with pregabalin 150, 300, and 600 mg/d, respectively, vs 54% of patients treated with PBO. Significant HRQoL improvements ($P \leq 0.05$) were observed in the following SF-36 domains: "bodily pain" (all doses), "vitality" (300, 600 mg/d), "mental health" (all doses), "social functioning" (300, 600 mg/d), "emotional-role limitations" (300, 600 mg/d), and "general health perception" (600 mg/d). Overall clinical status and HRQoL improvements appeared to be positively correlated with pregabalin dosage.
Conclusion: Findings from this analysis of a large patient cohort showed that painful DPN patients treated with pregabalin perceive significant improvement in their neuropathic pain and in their HRQoL in several domains of functioning.
Support: Pfizer Inc

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Comparison of Gabapentin and vitamin B_{12} therapy for symptomatic treatment of painful neuropathy in patients with type 2 diabetes mellitus
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Background and Aims: Approximately 45% of patients with diabetes have neuropathy during the course of the disease and pain is the main symptom. Vitamin B_{12} plays a vital role in synthesis of fatty acids for the maintenance of myelin sheath. Additionally it has an inhibitory role synthesis of TNF alpha, a potent nerotoxic cytokine while it promotes neurotrophic epidermal growth factor (EGF). We aimed to compare the effects and tolerability of two distinct agents, gabapentin and vitamin B_{12}, in painful diabetic neuropathy.
Materials and Methods: This was a single-centre, randomized, open, prospective, 12 week study. Patients attending to our diabetes outpatient clinic were screened according to following criteria: at least 30 years of age, no history of regular nonsteroidal anti-inflammatory drugs (NSAID) usage, normal complete blood count (CBC) and vitamin B_{12} (cobalamin) level, satisfactory metabolic control with present therapy (HbA1c <7.5%), presenting with clinically significant pain and paresthesia in the lower extremities for at least six months. 80 patients with the findings attributed to the loss of Achilles tendon reflex and/or loss of vibration sense, pain rating score of at least 40 mm on the 100-mm VAS and an average pain score of at least 4 on an 11-point Likert scale were recruited for the study. They were asked to complete the Short Form McGill Pain Questionnaire (SF-MPQ) and Short Form-36 Quality of Life (SF-36 QOL). They were allocated into two groups of 40. Group 1 received Gabapentin 1200 mg/day as starting dose for the first week and titrated to 2400 mg/day for the next 11 weeks. Vitamin B_{12} was introduced to Group 2 as 1000 mg/day parenterally for the first week and then subsequently given 3000 mg/day three times a week for

the remainder of the study. During the study dose of antidiabetic medication was not altered.
Results: 78 patients completed the study. Two were withdrawn from the gabapentin group because of reported dizziness. As the primary efficacy measurement is concerned; patients reported notable pain relief as they recorded their mean daily pain score at the end of the study. Gabapentin group had a baseline mean daily pain score of 6.8, improved to 4.1 at the end point of the study (p=0.001). Vitamin B_{12} group had a baseline mean daily pain score of 6.7 improved to 4.3 at the end point (p=0.001). There was no statistically significant difference between two groups at the end point.Effects of both drugs on secondary efficacy parameters were studied with SF-MPQ and SF-36 QOL questionnaire. Patients demonstrated significant improvement on the SF-MPQ; patients had significantly lower mean total pain and mean VAS scores at the end of the study (p=0.003), although no significant difference was observed between both groups. Apart from their effects on pain, both drugs had a similar effect on quality of life as the patients had a significant change at the end of 12 weeks. Positive effects of gabapentin were similar to Vitamin B_{12} as there was no significant difference between two groups.
Conclusion: These results suggest that vitamin B_{12} has comparable efficacy and tolerability to gabapentin in symptomatic treatment of painful diabetic neuropathy. Furthermore therapeutical aspects of vitamin B_{12} on fatty acid metabolism and neurotrophical factors, especially in the setting of insulin resistance could prove to be effective in prevention of progression of diabetic neuropathy.

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Effects of methylcobalamin on diabetic peripheral neuropathy: a systematic review of randomized evidence
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Background and Aims: To review systematically whether there is enough existing evidence that methylcobalamin is effective and safe in the treatment of the patients with diabetic peripheral neuropathy.
Materials and Methods: A Cochrane systematic review of all relevant randomized or quasi-randomized controlled trials of methycobalamin for diabetic peripheral neuropathy was performed. Clinical trials were searched from Cochrane Controlled Trials Register, Medline, Embase, The Chinese Biological Medicine Database, The Chinese Science and Technology Journal Full-text Database and references of all included trials. The selection of studies, data extraction and assessment of methodological quality were performed independently by two reviewers. The following outcomes were assessed: effectivenss of clinical symptoms and signs, sensory nerve and motor nerve conduction velocities and serious adverse events of methylcobalamin.
Results: Thirty randomized clinical trials including 1949 patients met the inclusion criteria. The quality of the most included trials was of low level. The "funnel plot" of the comparison of methylcobalamin plus some medicine with the same medicine showed that methylcobalamin showed asymmetry, which indicated possible publication bias and was related to low quality in methodology and small sample. The "funnel plot" of the comparison of methylcobalamin with other Vitamin B showed symmetry, which indicated less possible publication bias and the result was partly reliable. The results of Meta-analysis indicated that methylcobalamin showed significantly positive effects on the improvement of the symptoms and signs of peripheral neuropathy, and the effects are better than the other vitamin B. The increase of some nerves conduction velocities by methylcobalamin was better than by the other vitamin B. No serious adverse events were observed during the treatment period.
Conclusion: Methylcobalamin appears to be a safe and effective treatment on diabetic peripheral neuropathy. However, the evidence is not enough strong because some of the low qualities of trials and publications bias. Rigorously designs, randomized, double-blind, placebo-controlled trials of methylcobalamin for diabetic peripheral neuropathy are needed to further assess the effect.

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Effects of treatment with alpha-lipoic acid and benfotiamine in type 1 diabetic patients with peripheral neuropathy
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Background and Aims: The perspective direction in the treatment of diabetic peripheral neuropathy (DPN) is use of antioxidant drugs, and "neurotrofic" B-group vitamins in particular, benfothiamine-lisosoluble vitamin B_1. The aim of this open clinical trial was to evaluate the effects alpha-lipoic acid (ALA, the lipophilic free radicals "cleaner"), benfotiamine and their combination on the clinical course of DPN, dynamics of some biochemical parameters in type 1 diabetic patiens (type 1 DM) with DPN.
Materials and Methods: 61 patiens with type 1 DM and moderate/severe DPN (27,4 ± 3,2 years, 61 m), HbA1c 8,1 ± 1,3% were allocated to four group. Patients (pts) of group A (n=18) received ALA 600 mg in tab. tid; pts of group B (n=17) received BET 150 mg in dragee tid; pts of group C (n=15) received ALA 600 mg in tab. tid plus BET 150 mg in dragee tid and pts of group D (n=11) - placebo. The duration of the study was 2 months. Neuropathy Total Symptom Score-6 (NTSS-6-SA) and Neurological Disability Score (NDC) permitted us to assess positive sensory characteristic and stages of DPN. We also investigated the following parameters: superoxide dismutase (SOD), gluthation peroxidase (GPO) activities and reduced gluthatione (GSH), malondialdegide (MDA) contents in the RBCs', concentration of conjugated dienes (CD), ADP-induced platelet aggregation. Statitics: one way analysis of variance (ANOVA).
Results: It was established, that in type 1 DM patients and moderate/severe DPN the decrease of SOD (p<0,001), GPO activity (p<0,001), GSH content (p<0,001), the increase of MDA (p<0,001) and CD concentration (p 0,05); expessive of pain - 33% (group A), - 37% (group B), - 65% (group C); skin tenderness - 29% (group A), - 31% (group B), -59% (group C); paraesthesia - 27% (group A), - 34% (group B), -71% (group C); vibrational sensivity - 25% (group A), - 29% (group B), -58% (group C); numbness - 35% (group A), - 36% (group B), -74% (group C); tingling - 31% (group A), - 38% (group B), -69% (group C); others - 27% (group A), - 39% (group B), -61% (group C). Biochemical parameters: SOD activity - +46% (group A), + 51% (group B), + 68% (group C); GPO - from to 33% (group A), - and (group B), - and (group C). Simultaneously, more expressed increase of GSH content (p<0,01), GPO activity (p<0,05) and expressed decrease of thrombocytes aggregation parameters (degree of thrombocytes aggregation - 37%, p<0,01; stage of aggregation - 44%, (p<0,001), more exspessed decrease of MDA concentration (p<0,001) was marked in group C. In group D no positive clinical dynamics and dynamics of investigated parameters were revealed.
Conclusion: The combined therapy with ALA and benfothiamine of the type 1 DM patients with DPN during 2 months, was accompanied by more positive therapeutic effects, influence of the antioxidant state, platelet aggregation, what allows to recommend their use in the treatment of such patients.

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Skin blood flow and sensory thresholds change in type 2 diabetic neuropathic patients by α -lipoic acid infusion therapy
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Background and Aims: Antioxidants such as α -lipoic acid (ALA) have been shown to improve experimental DN. There are so many randomized clinical trials have evaluated the efficacy and safety of ALA in patients with DPN focusing on i.v. infusion treatment over weeks only by assessment of the Total Symptom Score or Neuropathy Impairment Score. But there is no clinical trials have evaluated the efficacy of skin blood flow change after i.v. infusion treatment over 7 days.
Materials and Methods: We recruited 14 diabetic patients with symptomatic DPN and 13 normal control subjects and evaluated the effects of ALA on skin blood flow change of DN patients by using Laser Doppler Flowmetry (Perimed®) during designed sequence of provocation tests. We measured sensory thresholds including vibration, cold thermal, and warm thermal thresholds using the VSA 3000 and TSA II (Medoc®) devices before and after treatment.
Results: Basal and provocated state blood flows in diabetic patients are lower than controls. In the arm skin blood flow significantly increased during cold pressor and limb lowering stimulation (p<0.05), but in the leg skin blood flow change did not difference before and after infusion therapy. Sensory thresholds of warm sense improved on the arm and leg, and cold

pain perception improved significantly ($p<0.05$) only on the arm. Vibration threshold did not show any difference before and after infusion therapy.
Conclusion: From this study, we suggest that ALA infusion therapy could improve small fiber function.

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Symptomatic diabetic peripheral neuropathy (SDPN) treatment with the PKC β inhibitor ruboxistaurin: trial design
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Background and Aims: Studies designed to evaluate symptomatic relief in diabetic peripheral neuropathy have largely focused on testing palliation of pain, over a 12-week period, with centrally acting agents. Based on the results of a phase 2 trial, we describe the study design for a phase 3 clinical trial evaluating the treatment of both painful and non-painful symptoms of diabetic peripheral neuropathy (DPN), with PKC β inhibitor, ruboxistaurin (RBX).
Materials and Methods: The study was a multicenter, randomized, double blinded, placebo controlled, parallel trial with two study arms. Patients with mild DPN received either RBX 32 mg/d or placebo for one year. Patients were identified by abnormal vibration threshold ([VDT]>95th percentile <23JND Units) and sural sensory nerve action potential >1 μV(SNAP+). From this patient pool, those with clinically significant symptoms (Neuropathy Total Symptom Score-6 [NTSS-6]>6) were selected. The primary objective of the study was to determine the potential of RBX to reduce the frequency and intensity of 6 painful and non-painful sensory symptoms of DPN as measured by the change in NTSS-6. Secondary objectives include evaluation of change in the neurological examination as measured by the neuropathy impairment score of lower limbs [NIS(LL)], quantitative sensory testing of vibration, and nerve conduction studies of peroneal, tibial and sural nerves. Patient demographic and baseline characteristics means were analyzed using a type I sum of squares analysis of variance (ANOVA).
Results: (Preliminary) 261 patients (137 male and 124 female) with type 1 (26.4%) or type 2 diabetes (73.6%) were randomized at 35 centers. The mean age was 47.1 ± 8.8 yrs; BMI was 29.9 ± 6.5 kg/m^2 and HbA_{1C} averaged 7.6 ± 1.4%. Patients had diabetes for a duration of 10.4 ± 9.2 yrs and duration of neuropathy was 2.7 ± 3.7 yrs. At baseline, over half of the patients used insulin therapy, nearly half used angiotensin-converting enzyme inhibitors/angiotensin II antagonists and approximately a quarter used palliative oral medicines. Baseline NTSS-6 total score was 9.5 ± 3.2 points (range: 3.67–19.82). Baseline NIS(LL) score was 7.2 ± 4.6 (range:0–38), and baseline VDT was 20.3 ± 2.2 JND units (range:11.3–23.8).
Conclusion: Limiting the study population to symptomatic neuropathy patients with pre-specified residual nerve function (SNAP+) resulted in randomization of a group of patients potentially more amenable to pharmacological intervention. This study population, in combination with 1) the use of a bidimensional instrument for assessment of neuropathic sensory symptoms and 2) extended study duration, may represent a unique opportunity to evaluate a sustained effect of a pharmacological agent acting on the peripheral nervous system.
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The diabetic foot: treating infection and releaving pressure

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Bacteria in superficial diabetic foot ulcers and their susceptibility to antibiotics through 10 years
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Background and Aims: Infection is a serious complication of diabetic foot ulcers and appropriate antibiotic therapy is an essential part of ulcer management. Development of antibiotic-resistant bacterial strains, triggered by the unnecessary use of wide-spectrum antibiotics, is a problem of increasing importance. The first-line antibiotic therapy for superficial diabetic foot ulcers in our centre since 1994 was amoxicillin/clavulanate (AM/CL). The aim of our study was to test whether this approach resulted in any change of antibiotic susceptibility.
Materials and Methods: The results of 107 diabetic foot ulcer swabs from the year 1994 and 65 swabs from 2004 were compared with respect to average number of isolated microrganisms, percentage of particular bacterial strains, and susceptibility to AM/CL, ciprofloxacin (CIP) and clindamycin (CLI).
Results: The number of isolated bacteria per sample in 1994 and 2004 was 2.06 and 2.71, p = 0.000. The percentage of isolated bacteria in 1994 vs 2004 was: Staphylococcus spp. 47.1 vs 39.3%; Streptococcus spp. 23.5 vs 19.1%, Enterobacteriaceae and nonfermentative gram-negative bacilli 19.5 vs 17.4%, Diphtheroids 3.2 vs 7.9%, Bacillus spp. 1.4 vs 0.6% and anaerobes 5.4 vs 14% (p=0.000). The susceptibility of Staphylococcus spp. to AM/CL decreased from 92.1% in 1994 to 81.4% in 2004, (p=0.001), but did not change with respect to CIP (88.9 vs 90.0%, p=0.770) and CLI (82.5 vs 81.4%, p=0.813). All tested Streptococcus spp. were sensitive to AM/CL in 1994 and 2004. In 2004, they were not tested to CIP. The sensitivity to CLI improved (54.5 vs 67.6%, p=0.000). The sensitivity of Enterobacteriaceae to AM/CL and CIP remained the same, and increased to CLI (4.4 vs 50%, p= 0.00). All tested anaerobes were susceptible to AM/CL, the susceptibility to CLI remained unchanged (73.7 vs 76%, p= 0.787), CIP was not tested in 2004.
Conclusion: The number of isolates per sample increased and the percentage of particular isolated bacterial strains changed significantly in the ten-year period. The routine use of AM/CL led to decreased susceptibility of Staphylococcus spp. to this antibiotic, whose efficiency against Enterobacteriaceae and anaerobes remains good. We therefore still recommend AM/CL as first line therapy for infected diabetic foot ulcers before definite microbiological results are available.

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Influence of inflammation on tissue penetration of linezolid in diabetic foot infections
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Background and Aims: Gram-positive aerobic bacteria still predominate in foot ulcers in diabetic patients. Methicillin-resistant staphylococci have emerged as a serious problem in patients with diabetic foot infections (DFI) with a prevalence up to 40% and the consequence of prolonged healing time and hospital stay. Linezolid could provide a useful therapeutic option in the treatment of gram-positive DFI, particularly those caused by resistant organisms. The aim of the study was to investigate the penetration of linezolid and penetration influencing factors in perinecrotic areals of infected diabetic foot wounds.
Materials and Methods: Tissue and plasma concentrations of linezolid (HPLC method) were evaluated at steady state (600 mg linezolid twice daily per os) 3 hours after application in 15 patients with diabetic foot infections (mean age: 63.1 ± 9.5 years; Wagner score 2/3). Penetration ratio (tissue concentration/corresponding plasma concentration) of linezolid was determinated. Correlations between tissue penetration of linezolid and clinical and laboratory parameters were evaluated (non-parametric Spearman-Rho correlations).

Results: With a mean tissue/plasma ratios of 101.7% (95% CI: 55.9; 147.6) linezolid achieved good penetration into perinecrotic tissue. This corresponds to a mean tissue concentration of 9.6 mg/kg (95% CI: 7.4; 11.8 mg/kg), which is above the concentration predicted to be effective against clinically important cocci (MIC_{90} for methicillin-resistant staphylococci 4 mg/L). Tissue/plasma ratios correlated with systemic inflammation (C-reactive protein: coefficient of correlation 0.73; p<0,01; serum albumin: -0.68; p<0,05; temperature: 0.6; p<0,05).
Conclusions: Linezolid achieves adequate levels in perinecrotic areals of diabetic foot wound. The penetration is positively correlated with inflammation. The data presented suggest that linezolid could be successful in the treatment of DFI.

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Does a diabetic foot infection (DFI) wound score correlate with the clinical response to antibiotic treatment? Data from the SIDESTEP study

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Background and Aims: A variety of scoring systems have been promulgated for classifying diabetic foot ulcers, and some have shown benefit in guiding treatment or predicting clinical outcomes. While several of these systems take infection of the ulcer into consideration, none are specifically designed for scoring the infected foot. We developed a DFI wound scoring system incorporating grading of both specific wound parameters (local and systemic signs and symptoms of infection) and wound size measurements. We sought to determine if the resultant composite DFI wound score determined at baseline and during treatment predicted the clinical outcome.
Materials and Methods: The SIDESTEP study was a multicenter, randomized, double-blinded comparison of intravenous (IV) ertapenem (E) (1 g/d) and piperacillin/tazobactam (P/T) (3.375 g qid) for treatment of patients with moderate to severe DFI. Investigators graded each infection for the presence of purulent drainage, non-purulent drainage, erythema, induration, tenderness, pain, and local warmth for severity with a value ranging from 0 (absent) to 3 (severe). They also measured wound size, area, and undermining with scores ranging from 0 to 10 for size and depth, and 0 to 8 for undermining. The total DFI wound score, defined as the sum of the individual scores, was calculated at baseline, discontinuation of IV therapy (DCIV) and at a follow-up assessment (FUA) 10 days after cessation of antibiotic therapy.
Results: At baseline, DFI wound scores were calculated for 92.8% (373/402) of clinically evaluable patients; mean 16.1 ± 5.6 for E and 15.6 ± 5.7 for P/T. E treatment group: the favorable clinical response (infection improved or cured) at FUA ranged from 100% (52/52) in patients with a baseline wound score ≤ 12 to 81.8% (36/44) in patients with a baseline wound score >19. P/T: the favorable clinical response at FUA ranged from 90.6% (58/64) in patients with a baseline wound score ≤12 to 72.1% (31/43) in patients with a baseline wound score >19.
Conclusion: Mean DFI wound scores were similar for the two treatment groups in this study. Clinical response rates to antibiotic therapy were similar in both treatment groups at baseline, DCIV and FUA and the response rates generally decreased with an increase in baseline wound score. The DFI wound score may be a useful tool for assessing wound infection severity and in predicting treatment outcomes.

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Type 1 diabetes associated with a high prevalence of low bone density

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Background and Aims: Recent literature has suggested a possible association between type 1 diabetes (DM 1) and low bone mineral density (BMD). However, current diabetes guidelines (ADA, CDA) and osteoporosis guidelines (NOF, OSC) make no mention of this potential association despite the fact that some studies show up to a twelve fold increased risk of osteoporotic fractures. To expand upon the limited literature on this topic and to determine if such an association exists in adults, we surveyed adult patients with DM 1 from a large community diabetes practice.
Materials and Methods: All active patients aged 20 to 65 with DM 1 not meeting exclusion criteria (pregnancy or actively seeking pregnancy; known secondary cause of osteoporosis/low BMD; use of anti-resorptive medication, unable or unavailable to participate) were eligible for inclusion (267 patients). One hundred and thirty patients completed a standardized questionnaire and a BMD assessment.
Results: Of the130 who completed the standardized questionnaire and BMD assessment, 52 (40%) had a T score of < -1.0 in at least one of three measured sites (femoral neck, total hip, lumbar spine). Men were more likely to be affected (29 of 63; 46%) than women (23 of 67; 34%), particularly in the quartile with the lowest BMD (21 men of 32 individuals; 65%). Low BMD was more common in older age groups (20 of 37 aged 50–65; 54%), but still notably high among younger age groups (11 of 30 aged 20–34; 37%). Traditional osteoporosis risk factors did not seem helpful in discriminating the quartile with the lowest BMD from that with the highest with the exception of prevalent fracture which was more common in those with the lowest BMD scores; however lower body mass index, longer duration of diabetes, and male gender were associated with lower BMD.
Conclusion: This data suggests a significant proportion of adults with DM 1 have low BMD with a disproportionate representation of men in the most severely affected group. Further study is needed to assess the mechanism behind both the increased prevalence of low BMD observed in persons with DM 1 and the factors leading to the over-representation of affected males.

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In Charcot osteoarthropathy early offloading prevents bone mineral density falls not only in the Charcot foot but in the contralateral non-Charcot foot

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Background and Aims: The aim of this study was to measure prospectively calcaneal bone mineral density (BMD) in the Charcot and non-Charcot foot in the acute and chronic stages of Charcot osteoarthropathy.
Materials and Methods: We studied 31 patients with acute unilateral Charcot osteoarthropathy: 20 patients presenting with established x-ray changes and 11 patients with normal x-ray but abnormal bone scan. All patients were treated with offloading.
Results: In patients with x-ray changes, BMD was significantly reduced in the Charcot foot compared with the non-Charcot foot (0.391 ± 0.12 g/cm^2 versus 0.477 ± 0.13 g/cm^2, p<0.001). On follow up over 10 ± 4 months despite offloading, there was significant reduction in BMD not only in the Charcot foot (0.353 ± 0.11 g/cm^2, p=0.034) but also in the non-Charcot foot (0.456 ± 0.13 g/cm^2, p=0.008).
In contrast, patients presenting with normal x-ray, had similar BMD between the Charcot foot and non-Charcot foot (0.412 ± 0.11 g/cm^2 versus 0.428 ± 0.1 g/cm^2, p=0.438). On follow up over 8 ± 2 months, after offloading BMD did not fall either in the Charcot foot (0.416 ± 0.12 g/cm^2, p=0.795) or in the non-Charcot foot (0.439 ± 0.1 g/cm^2, p=0.261).
Conclusion: Offloading in Charcot osteoarthropathy may prevent falls in bone mineral density in the Charcot foot and non-Charcot foot if applied promptly before radiological changes have developed.
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Risk and benefit of total contact cast treatment of the diabetic foot related to osteomyelitis

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Background and Aims: Total contact cast (TCC) is one of the most suitable lower-limb off-loading methods used in diabetic foot treatment. TCC therapy may improve healing of existing osteomyelitis (OM). On the other hand, the risk of this method is that it could also induce progression of infection and development of a new OM. The aims of our study were to assess risk vs. benefit of TCC treatment related to OM and factors influencing healing of OM and development of a new OM.
Materials and Methods: 61 unselected patients (mean age 54 ± 9 years, diabetes duration 15.6 ± 9 years, HbA1c 8.3 ± 1.8%) treated in our foot clinic for the diabetic foot by removable TCC from 2/2002 to 12/2003 were included into our study. Patients were observed and treated by TCC until healing of the diabetic foot or at least for 2 months and maximum for 12 months (removable TCC was applied for 5.4 ± 4.5 month on average in all patients). OM was diagnosed by X-ray and laboratory markers of infection

(CRP, Leukocytes). The frequency of patients with OM before therapy and healed after TCC application was compared with the frequency of patients with newly developed OM during TCC therapy (McNemar test). Factors influencing healing of OM such as OM location, the type of ulcer and microbial findings were assessed. The rates of all complications were recorded during the observed period.
Results: 21/61 (34%) of patients had OM before TCC therapy and 11/21 (52%) of them healed during TCC treatment. From 40 patients without OM before TCC application, 3 patients (8%) developed new OM during TCC therapy. The frequency of patients with healed OM was significantly higher compared with the frequency of patients with newly developed OM ($p<0.01$), it means that the benefit of TCC therapy on healing of OM was 3.6 times higher in comparison with the risk of a newly developed OM during application of this off-loading method. 10 patients with previously diagnosed non-healed OM were characterized primarily by OM location in tarsal bones; other factors such as the type of ulcer and microbial resistance had no significant effect. Patients with a newly developed OM were specified by ulcer of Wagner 3 and infection caused by resistant pathogens. The most common other complication of TCC therapy in the study group was broken cast (49%), followed by the development of a new neuropathic ulcer on the same foot (25%). Progression of local infection leading to discontinuation of TCC therapy was noted in 11% of all patients. Other complications such as joint pain (2%) and mycosis (3%) were seen very rarely.
Conclusion: Our data showed that benefit of TCC therapy on healing of OM was significantly higher in comparison with the risk of a newly developed OM during this treatment. However, application of TCC in patients with OM located in tarsal bones and in patients with neuropathic foot ulcers of Wagner 3 and/or with infection caused by resistant pathogens is still controversial.
This study was supported by the Institute for Clinical and Experimental Medicine (MZO 00023001).

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How long does it take to progress from cast to shoes in the management of Charcot osteoarthropathy?
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Background and Aims: Charcot osteoarthropathy can be managed either with total contact cast or removable cast walker. However, the transition from cast to bespoke footwear is not well described. We report the duration of casting in the management of 46 patients with Charcot osteoarthropathy and we emphasize that over 30% of patients relapsed after coming out of a cast.
Materials and Methods: We studied 23 type 1 and 23 type 2 diabetes with acute Charcot osteoarthropathy. Mean age was 50 ± 13.1 years (mean±SD) and mean duration of diabetes was 20 ± 11.1 years. Total contact cast was applied in 34 patients as the primary treatment but when there were contraindications to it, a removable cast walker was used in 12 patients. Patients were changed either from total contact cast to bivalve cast and then bespoke shoes or from removable cast walker to shoes when foot skin temperature difference was less than 2°C and there was a radiographic evidence of consolidation. Relapse was defined as an increased foot skin temperature difference of greater than 2°C after the primary casting treatment had been concluded.
Results: The median duration of casting treatment for all patients was 11 (8–16.7) months [median (25^{th}–75^{th} percentile)]. Thirty-one patients (67%) progressed from the primary treatment to bespoke footwear without relapse and duration of casting was 9 (7–12) months. However, 15 patients (33%) relapsed after completing their primary casting treatment. They had to return to their casting treatment and the total duration of casting for these patients was 20 (15–21) months, ($p<0.001$). Relapse of Charcot osteoarthropathy was not correlated with the type of primary casting treatment ($r=0.220$ $p=0.141$), age ($r=0.082$, $p=0.587$), duration of diabetes ($r=0.182$, $p=0.226$), type of diabetes ($r=0.046$, $p=0.76$), gender ($r=0.016$, $p=0.915$) and site of involvement ($r=0.07$, $p=0.627$).
Conclusion: This study has shown that the total duration of casting is longer than previously reported and emphasizes that over 30% of the patients relapse after completing their primary cast treatment. It is important that patients with Charcot osteoarthropathy should be observed very carefully to identify relapse early.

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Nonremovable fiberglass off-loading walking cast versus aircast in the treatment of neuropathic foot ulcers: a controlled randomized clinical study
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Background and Aims: Neuropathic plantar ulcers are the most frequent complication of sensitive motor diabetic neuropathy. The altered plantar bearing, consequent to articular rigidity and to the foot structural deformities, is responsible for the appearance of plantar peaks of pressure which condition the rise of non-painful areas of callosity, frequently evolving into ulcerative wounds.The treatment of choice of neuropathic plantar ulcers is given by the total off-loading of the ulcers, achieved with an off-loading device. Our group has recently shown the superiority of the nonremovable fiberglass off-loading walking cast (OWC) vs. a therapeutic shoe with off-loading insole. The complexity of preparing such an off-loading device has not allowed a mass usage of this technique. Purpose of the study is the comparison of efficacy and safety of a new removable off-loading device (AIRCAST).
Materials and Methods: We consecutively enrolled 36 patients affected by plantar ulcers with at least 3 months history, with an ulcer surface ≥ 1 cm^2 and TcP02 ≥ 40 mmHg. Exclusion criteria were: clinically infected wound, bone exposure, x-ray confirmed osteomyelitis, contra-indications to the use of the off-loading device (reduced visual acuity, impaired equilibrium, amputed controlateral limb). 19 patients were enrolled in the OWC group while 17 in the Aircast group. The ulcer surface was 370 ± 340 mm^2 in the OCW group and 240 ± 170 in the AIRCAST group. The study lasted 90 days. The control visits were done every 12 days at the Center. During the visit the OCW or Aircast was removed, the wound was dressed (surgical couretage, cleansing with iv sodium solution, application of hyaluronic acid, polyurethane foam) and the OCW remade or the AIRCAST re-applied. The patients from the Aircast group were informed of the need to wear continuously the device in order to obtain ulcer healing.
Results: At the end of the 90-day observation period 16 patients (81%) in the OCW group healed, with an average time of 43.3 ± 19.5 days, while in the Aircast group 13 pts (76%) healed, with an average of 53.3 ± 20.5 days. In the OCW group there were 3 dropouts, while in the AIRCAST group there were 4.
Conclusion: Data coming out from our study demonstrated that there are not statistically differences between the two treatments both from efficacy and safety point of view. We can state that AIRCAST device can be considered a good off-loading system for the treatment of neuropathic plantar ulcers.

PS 90

Inflammation, apoptosis and the neuropathic foot

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Abstract withdrawn

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Sustained elevated serum concentrations of the proinflammatory cytokine IL-6 in type 2 diabetes patients with diabetic foot syndrome

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Background and Aims: The development of a diabetic foot syndrome (DFS) is a common complication of diabetes accompanied by strong impairments of life quality due to lower limb amputation. An association with immunological processes has not been described yet. Therefore, we analysed serum levels of immunological parameters in type 2 diabetes patients with and without DFS to test the hypothesis that alterations of immune parameters are associated with DFS.
Materials and Methods: 151 patients with type 2 diabetes were included in this study. 40 patients (14 women, 26 men) had active foot ulcers (classified according to Wagner, grade 0–4), 111 control patients (50 women, 61 men) were without history of previous foot lesions. Blood samples were drawn at hospitalisation, after 7 and 14 days, and serum was analysed for the acute phase protein CRP by high sensitivity latex enhanced nephelometric assay and for the proinflammatory cytokine interleukin-6 (IL-6) using high sensitive ELISA. Patients were intensively evaluated for clinical parameters including wound size, infection grade and diabetes-related complications. Both groups were only slightly different regarding age (Mean +/– SD 66.0 +/– 9.0 vs. 62.5 +/– 8.5 years, p=0.03).
Results: At hospitalisation, patients with DFS exhibited significantly elevated levels of IL-6 and CRP compared to controls (median 10.2 pg/ml vs. 3.8 pg/ml, p<0,0001 for IL-6 and 11.0 mg/l vs. 2.6 mg/l for CRP, p<0,0001). Whereas CRP levels declined during the first 14 days of intensive therapy along with clinical signs of infection and wound size, IL-6 levels persisted during the observation period (day 0: median 10.2 pg/ml vs. day 14: median 8.6 pg/ml; p>0,05). For initial values of both, IL-6 and CRP, a strong correlation with severity of foot ulcer as assessed by Wagner (r=0.50, p<0.01 for IL-6, r=0.55, p<0.001 for CRP) and duration of hospitalisation was observed. Interestingly, CRP but not IL-6 was associated with the grade of infection of foot ulcer (r=0.18, p=0.27 for IL-6, r=0.34, p<0.05 for CRP).
Conclusion: Elevated serum levels of the proinflammatory cytokine IL-6 were observed in type 2 diabetes patients with DFS compared to patients without history of DFS. The IL-6 levels persisted independently of clinical outcome and adequate antiinfective therapy. Additionally, patients with severe ulcers exhibited a stronger immune activation and a tendency towards longer hospitalisation periods. These results suggest an influence of innate immunity in the pathogenesis of diabetic foot syndrome independent of the acute phase response.

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Cytokine analyses in wound fluids of the diabetic foot syndrome reflects inflammation and the various healing stages

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Background and Aims: Defects of wound healing on a cellular level in patients with diabetes mellitus can be explained by disturbed granulocyte function, which in particular relates to the altered release of mediators of wound healing and growth factors. In addition, the inflammatory action appears to be affected by systemic disturbances of diabetes and altered cellular processing. In this study cytokines (IL-6) and growth factors (TNF) were investigated in wound fluids in relation to wound size, wound healing duration, bacterial load and systemic parameters of inflammation (CRP, leukocyte count).
Materials and Methods: 22 diabetic patients (10 men, 12 women; mean age 67 ± 19 years; HbA1c 7.7 ± 1,4) with the diabetic foot syndrome (stage Wagner 2 and 3) were studied. The wound fluids were washed with 5 ml of NaCl solution and were collected after changing the wound dressing and were stored at -20°C. Microbiological analyses of deep swabs were performed and correlated to cytokines and clinical staging.
Results: We did not find differences in wound size, glycemic control, duration of wound healing or patients' age between diabetic patients with and without a positive microbiological result. There was a clear and significant correlation between TNFalpha and the wound size. There were no differences for CRP or leukocytes between these two patient groups. However, TNFalpha (28 vs. 23 pg/ml: p=0.012) and IL-6 (367 vs. 42 pg/ml; p=0.03) were significantly increased when in the state of infection. This was independent of the clinical stage of wound scoring.
Conclusion: This study demonstrates a strong correlation of TNFalpha and IL-6 with the microbiologically determined infection. Additionally, there was a correlation of TNFalpha and wound size. These data demonstrate that these markers represent a reaction to bacterial wound infection.

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12/15-lipoxygenase and early diabetic neuropathy

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Background and Aims: Growing evidence indicates that upregulation of the enzyme of arachidonic acid metabolism, 12/15-lipoxygenase (LO), is an important factor in the pathogenesis of diabetic complications. Increased 12/15-LO expression and activity contribute to oxidative stress, MAPK activation, inflammation, and altered transcriptional regulation in diabetic cardiovascular disease and nephropathy. The present study was aimed at evaluating the role for 12/15-LO in early peripheral diabetic neuropathy (PDN).
Materials and Methods: Adult male wild-type (LO+/+) and 12/15-LO–/– mice have been made diabetic with STZ (100 mg $kg^{-1}d^{-1}$ i.p., several injections were needed for some LO–/– mice). Diabetic mice have been divided into two groups i. e. with moderate and severe hyperglycemia (blood glucose ~20 mM and ~30 mM, respectively). Motor and sensory nerve conduction velocities (MNCV and SNCV), thermal algesia, and tactile response thresholds have been evaluated 10 wks after induction of diabetes. Then control (C) and STZ-diabetic (D) wild-type mice were treated with/without the 12-LO and 12/15-LO inhibitor cinnamyl-3,4-dihydroxy-alpha-cyanocinnamate (CDC, 8 mg $kg^{-1}d^{-1}$ s.c.) for another 10 days, after which nerve functional measurements have been performed again.
Results: Despite similar blood glucose levels, D LO-/- mice had much less severe PDN than D LO+/+ mice. In particular, SNCV was reduced by 14% and 16% (p<0.01 vs C for both) in D LO+/+ mice with moderate and severe hyperglycemia, and by 5% and 11% in D LO-/- mice with similar blood glucose levels (p >0.05 and p < 0.01, respectively). In the group with moderate hyperglycemia, MNCV was reduced by 18% in D LO+/+ mice (p<0.01 vs C), whereas D LO–/– preserved normal MNCV. In the group with severe hyperglycemia, MNCV deficit achieved 29% in D LO+/+ mice (p<0.01), but only 5% in D LO–/– mice. Tactile response thresholds decreased by 83% in D LO+/+ mice with moderate hyperglycemia (p<0.01 vs C) consistent with clearly manifest tactile allodynia; the corresponding decrease was equal to 25% in D LO–/– mice (p<0.05 vs C). In the group with severe hyperglycemia, tactile response thresholds decreased by 68% and 27% in D LO+/+ and D LO–/– mice (p< 0.01 and <0.05 vs C, respectively). Thermal response latencies increased by 48% and 32% in D LO+/+ and D LO–/– mice with moderate hyperglycemia (p<0.01 and <0.05), consistent with the development of thermal hypoalgesia in both groups. This increase progressed to 97% and 40% in D LO+/+ mice and D LO–/– mice with severe hyperglycemia (p<0.01 vs C for both). CDC treatment partially reversed SNCV and MNCV deficits and tactile allodynia, but not thermal hypoalgesia, in STZ-diabetic wild-type mice. Body weight and blood glucose concentrations in either control or diabetic mice, and any functional variables in control mice were not affected by the LO inhibitor treatment.
Conclusion: The 12/15-LO pathway plays an important role in nerve conduction deficits, abnormal sensation and pain associated with early PDN. Mechanistic studies in cell culture models are in progress.

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Poly (ADP-ribose) polymerase-1 gene (*PARP1*) contributes to the genetic predisposition to diabetic polyneuropathy in Russian patients with type 1 diabetes

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Background and Aims: Oxidative stress has been suggested as one of the main causes of diabetic polyneuropathy (DPN) in patients with type 1 diabetes mellitus (T1DM). Earlier we have found an association of three genes (*SOD2*, *SOD3* and *CAT*), encoding antioxidant scavenging enzymes, with DPN in Russian patients with T1DM. Poly (ADP-ribose) polymerase-1 (PARP-1) functions as a DNA damage sensor and signaling molecule binding to both single- and double-stranded DNA breaks. ADP-ribosilation facilitates DNA repair and thus permits cell survival. Severe DNA damage, however, leads to overexpression of PARP, resulting in NAD^+ and ATP depletion and necrotic cell death. We propose that *PARP1* gene, encoding important DNA damage sensor - PARP-1 enzyme, can be involved in the genetic susceptibility to DPN. To examine this hypothesis we developed the new polymorphic markers (*Leu54Phe* and *Val762Ala*), located in the coding area of *RARP1* gene, and studied an association of these markers with DPN.
Materials and Methods: A case-control study was carried out in a group of 179 unrelated Russian patients with T1DM, 86 of whom had overt DPN (DPN+ group) and 93 had no clinical DPN (DPN- group). To identify the polymorphic marker alleles we used PCR technique in combination with restriction endonuclease cleavage and gel electrophoresis.
Results: Both markers have shown a strong association with DPN. In case of *Leu54Phe* marker we have shown that the carriers of *Leu* allele (*OR* = 0.60, CI = 0.39–0.92; *p* = 0.023) and *Leu/Leu* genotype (*OR* = 0.41, CI = 0.20–0.85; *p* < 0.019) had lower risk, whereas the carriers of *Phe* allele (*OR* = 1.66, CI = 1.08–2.54; *p* = 0.023) had higher risk of DPN development. Polymorphism *Leu54Phe* locates in area of zinc fingers, which are responsible for DNA binding and some protein-protein interaction, can influence on the effectiveness of DNA binding or protein interactions. In case of *Val762Ala* marker we have shown that the carriers of *Val* allele (*OR* = 0.35, CI = 0.17–0.70; *p* = 0.002) and *Val/Val* genotype (*OR* = 0.42, CI = 0.19–0.92; *p* <0.037) had lower risk, whereas the carriers of *Ala* allele (*OR* = 2.88, CI = 1.43–5.77; *p* = 0.002) had higher risk of DPN development. Polymorphism *Val762Ala* locates within or nearby the active centre of PARP-1 enzyme and can influence on its catalytic activity.
Conclusion: The results of our study are evidence that *Leu54Phe* and *Val762Ala* markers, located in the coding area of *RARP1* gene, are strongly associated with DPN in Russian patients with T1DM. These data support a hypothesis concerning an involvement of *PARP1* gene into the formation of genetic susceptibility to DPN.

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Diabetic peripheral neuropathy: Evidences for ventral horn neurons apoptosis

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Background and Aims: Diabetes Mellitus (DM) affecting at least 171 000 000 people around the world by the year 2000, is considered a life-threatening condition. This disease and its complications contribute substantially to world premature adult mortality rates, in a similar way as the worrisome modern HIV/AIDS epidemic. In addition, diabetes prevalence is expected to growth more than double in a 30 years period, mostly affecting the poorest in the population. Diabetic neuropathy is at the same time one of the most frightening complications of DM and the most common form of peripheral neuropathy. This complication pathophysiology is still not well understood. In great extent the experimental studies focus on sensory damage, neglecting the motor component. Motor nerve damage in diabetes is a long term, highly incapacitating, and life quality threatening secondary condition. It represents elevated social costs. However, studies on motor nerve cells pathophysiological mechanisms associated to diabetes are scarce. Recently, it has been demonstrated that peripheral sensory neuropathy is associated with apoptosis. Our aim was to evidence the participation of the apoptotic mechanism in the diabetic motor neuropathy.
Materials and Methods: We used the streptozotocin (STZ) induced diabetic rat model. In brief, 6 weeks old, male, 12 h fasted Wistar rats were i.p. injected with STZ at 70 mg/Kg. Weight and blood glucose levels were tested before, as well 24, 72, and 120 h following diabetes induction. Under urethane anesthesia (1 gr/kg, i.p.), the sciatic-tibial motor nerve conduction velocity was also assessed before and after (48, 96 and 144 h) STZ injection. One week after confirmation of hyperglycemia, under urethane anesthesia the rat medulla of both experimental and control animals were quickly (<90 seconds) removed. Then, the medulla was perfused with oxygenated Ringer solution, transversally sectioned and the anterior horns of the dorso-lumbar region were sectioned in thin 1–2 mm blocks. These samples were subjected to Trypan blue cell viability, Bcl2 and Bax RT-PCR, and inmunohystochemistry (Terminal dUTP Nick-End Labeling –TUNEL–) tests.
Results: Weight, motor nerve conduction velocity and blood glucose levels showed consistent changes in the diabetic induced rats: reduction in the two first parameters and increase >250 mg/dl in the latter. The dorsal horn nerve cells viability test exhibited a reduction in the STZ group when compared to the control group, 79.8 vs 95.8%; p = 0.004. In the RT-PCR experiments, both control and diabetic neurons showed over-expression of the pro-apoptotic protein Bax, although a greater per cent increase was documented in the STZ-treated samples, p = 0.03. Bcl2 resulted no different between the diabetes and control groups, p = 0.06. The TUNEL test evidenced a highly significative difference between the TUNEL (+) nerve cells in the ventral horn of diabetic rats when compared to controls (p < 0.001).
Conclusion: Our results evidenced the apoptotic mechanism affects the ventral horn nerve cells in STZ-diabetic rats exhibiting peripheral motor neuropathy.
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Neurogenic responses to skin heating (LDIflare) and mechanical trauma in type 2 diabetes

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Background and Aims: We recently described, a novel, simple, non-invasive method, the LDIflare, which demonstrates impaired C-fibre function in type 2 diabetes prior to detection of neuropathy by other methods. This involves heating skin on the dorsum of the foot to 44^0 C and measuring the neurogenic flare using a scanning laser Doppler. We compared this response with the flare induced by mechanical injury resulting from a 3 mm skin biopsy in the same area (Biopsyflare).
Materials and Methods: Twelve healthy controls (HC) and 12 type 2 subjects with neuropathy (DN) without macrovascular disease were studied.
Results: The LDIflares in HC and DN were 5.18+/–1.80; 1.80 +/– 0.67 (mean +/– Std. dev in cm^2) respectively. The Biopsyflare in the same groups were 0.78 +/- 0.3; 0.37 +/– 0.14 respectively. The LDIflare and Biopsy flare were significantly reduced in the diabetic neuropathic group. (p < 0.0001; p= 0.01 respectively). Although the Biopsy flare was significantly smaller in both groups compared to the LDIflare the two injury responses correlated well. (r = 0.59, p <0.0001).
Conclusion: This study confirms that the neurovascular responses to two different noxious stimuli are reduced in subjects with Type 2 diabetes, and have good correlation. Thus, the LDIflare being non-invasive should be the preferred method of assessing the neurogenic response to tissue trauma.

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The diabetic foot and angiopathy

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Close temporal association between established renal failure and the incidence of both foot ulcers and major amputation in diabetes mellitus

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Background and Aims: Established renal failure is associated with a substantial risk of peripheral vascular disease, and this is most marked in diabetes mellitus. Such disease is typically associated with foot ulceration and with amputation. While the reasons for the association between vascular disease and diabetic nephropathy are multiple, it has been suggested that there may be a close temporal association between the incidence of amputation (and of foot ulcers), and the start of renal replacement therapy
Materials and Methods: We examined the detailed databases kept by both the Department of Renal Medicine and the Foot Ulcer Trials Unit of the Department of Diabetes and Endocrinology, to determine the extent of any such association.
Results: Of 2626 patients who have been treated with maintenance dialysis at the City Hospital, Nottingham since 1978, there were 466 (17.7%) who had diabetes. 94 (20.2%) of these 466 were also recorded as having been managed in the Department of Diabetes and Endocrinology with a foot ulcer, and 18 (19.1% of 94) of those with an ulcer underwent major amputation. The unadjusted cumulative incidence of first recorded foot ulceration rose steeply in the period between 500 days prior and 1500 days post the onset of renal replacement therapy, with 64 (68.1%) occurring in this time, as did 9 (56%) of all major amputations. The increase in incidence after the start of treatment was greater in those managed with haemodialysis, as opposed to peritoneal dialysis although those treated with haemodialysis were older ($F_{(1,461)}$=39.90, p=<0.001), and had a higher prevalence of type 2 diabetes (χ^2=22.14, df=1, p<0.001).
Conclusion: The reasons for the association between the onset of dialysis and the steep rise in incidence include generalised ill-health, anaemia, worsening vascular disease, changing priorities of overall care – as well as the possibility of a direct adverse effect of dialysis on peripheral limb oxygenation. Designated foot protection services should be made available to all patients on dialysis. This is particularly important around the time that dialysis is started when the risk of ulceration (and hence of amputation with associated high mortality) is greatest, and should be an essential feature of planned care.

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Study of the effect of haemodialysis on transcutaneous oxygen tension ($TcpO_2$) in the lower limb of patients with diabetes

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Background and Aims: The onset of haemodialysis in patients with diabetic nephropathy has been reported to be associated with the development of lower limb critical ischaemia and limb loss. The high incidence of limb loss is not a feature of diabetic nephropathy prior to the onset of end-stage renal failure. Although the factors underlying any such association are multiple, it is possible that the fluid shifts and haemodynamic responses inherent in the process of haemodialysis may result in temporary worsening of the peripheral circulation and may trigger peripheral ischaemia in those already predisposed to it. This study was designed to test the hypothesis that haemodialysis adversely affects tissue oxygenation in patients with diabetes.
Materials and Methods: Ten diabetic patients who were being managed with maintenance haemodialysis gave informed consent to participate in the study. $TcpO_2$ was measured using a multichannel TCM400 (Radiometer, Copenhagen). Measurements were made continuously from the dorsum of both hands and of one foot, starting before haemodialysis and for a period of four hours afterwards.
Results: Median (IQ range) was 73 (69–77) years. There were seven men, and nine of the 10 had type 2 diabetes. All were free from active ulceration, and had no history of peripheral limb ischaemia. Following equilibration median baseline $TcpO_2$ was 54.5 mmHg. At the end of the period of dialysis, the median was 54.0. Median $TcpO_2$ at one, two and four hours after the end of dialysis was 50.0, 49.0 and 47.0 mmHg, respectively. Analysis by ANOVA suggested that there was a trend towards a difference between the groups (p=0.066). Oxygen tension did not fall at the other measured sites.
Conclusion: This study suggests the process of haemodialysis is associated with a fall in tissue oxygenation. It is possible that such an effect may be more pronounced in those with clinical signs of significant vascular disease, and may be a factor which contributes to the temporal association between the onset of dialysis and the apparent steep rise in the incidence of foot ulcers and amputation.
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Angiogenic potential in patients at very high risk for foot ulceration

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Background and Aims: Diabetic patients with high VPT are at risk of foot ulceration. Angiogenesis is essential for foot skin repair. We investigated the angiogenic potential in high risk diabetic patients.
Materials and Methods: We studied the skin of the foot in terms of (a) microvascular reactivity (b) immunohistology in a group of non vascular diabetic patients with VPT=>99pc (HR) compared to diabetic patients with VPT<99pc (D) and normal controls (C).
Results: We found alterations in microvascular function (ACh response 205+/–91% in HR vs. 485+/-226 in D and 797+/–220 in C, ANOVA p<0.05) but not structure, and an increased expression of hypoxia-inducible factor 1-alpha (21+/–8 in C, 54+/–30 in D, 97+/-44 in HR, arbitrary units) as well as of VEGF-A+BVD (52+/–11 C, 45+/-9 D, 68+/–21 HR No/sq.mcm) and VEGF-R2+Blood vessel density (29+/–8 C, 52+/–14 D, 57+/-31 HR No/sq.mcm). Correlations are found between ACh response and VEGF intensity (Spearman's r=0.448 and P=0.028) and between HIF1-alpha intensity and VEGFR2+BVD (Spearman's r=0.581, P=0.003). Overall (von-Willenbrand Factor+) blood vessel density did not differ significantly between groups although tended to increase in HR (259+/–41 HR vs. 240+/–26 D and 205+/–24 C, No/sq.mcm).
Conclusion: Hypoxia appears to be crucial in the diabetic foot complications and can be present even in the absence of clinical macrovascular disease. Physiological VEGF response may not be sufficient to guarantee an adequate neovascularisation, essential for wound repair.

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Distal angioplasty in diabetic foot syndrome: long term outcomes

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Background and Aims: The prevalence of peripheral vascular disease is 20–30% in adult diabetic population. In diabetes peripheral vascular disease involves specifically the distal arterial tree, in particular arteries below the knee. In the last years percutaneus angioplasty (PTA) became the main revascularization procedure in lower limb salvage in diabetic foot syndrome. Aims of this study is to evaluate in diabetic patients with diabetic foot syndrome revascularized by PTA: 1) the clinical outcome (precence or absence of major amputation); 2) the patency of treated arteries (with ultrasound duplex tecnique); 3) transcutaneous oxygen tension before-PTA, after-PTA and at follow-up (507 ± 272 days, mean±SD).
Materials and Methods: Between January 2002 and January 2004 in the Magenta Hospital, Italy, 30 PTA of below the knee district, in diabetic patients with ulcer, necrosis or gangrene and TcpO2 <40 mmHg, and without haemodinamic alteration of proximal arterial tree were performed.
Results: 1) At follow-up 5 patients died, 1 had major amputation. 2) Duplex ultrasound showed almost one vessel patency in 17 patients (75%) (group I), the others 7 patients (group II) had restenosis >50% or occlusion. 3) oximetric values increased significantly immediately after PTA (32 ± 6.3 mmHg vs 10 ± 7.2 mmHg before PTA, p<0.001) and at follow-up (53.8 ± 12.2 mmHg, p<0.001). Oximetric values before-PTA were significantly higher in patients that at follow-up had patent arteries than in patients with restenosis (group I: 20.5 ± 13 mmHg, group II: 10 ± 7 mmHg, p= 0.03) while at follow-up oximetric values weren't significatively different in the two groups (62.3 ± 13 mmHg vs 53.8 ± 12 mmHg, p=0.1). Not differences were founded between the two groups for cardiovascular risk factors: age, diabetes onset, follow-up time, antiplatelet therapy.

Conclusion: These results: 1) confirm PTA efficacy in limb salvage (96%); 2) demonstrate long term arterial patency post distal PTA; 3) show the prognostic value of oximetric levels pre-PTA on long term arterial patency; 4) show that oximetric values at follow-up aren't influenced by restenosis.

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Screening for peripheral arterial disease in a primary health care population of patients with diabetes mellitus, a six year follow up study

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Background and Aims: The development of reduced peripheral blood flow in primary care populations of patients with the diagnosis diabetes mellitus, has not been studied since strain gauge methodology, the golden standard method, is expensive and not suited for epidemiological studies. The aim of this study was to compare the ability to detect peripheral arterial disease between traditional ankle Doppler technique for measuring ankle blood pressure (ADP) and a new pulse oximetric method for measuring systolic toe pressure (STP), and study the change in peripheral blood flow during six years in a primary health care population with diabetes mellitus.
Material and methods: 111 patients with the diagnosis diabetes mellitus in two primary health care districts were studied at the start of the study and again after six years. The population was investigated by means of pulse palpation, ADP, STP and arm blood pressure. After six years all measurements were repeated. Mortality and death causes in the investigated population was studied.
Results: In the 111 patients included, 221 extremities were investigated during the first study and 173 extremities were reinvestigated after six years. 24 patients died during the follow up period. ADP and ankle/brachial pressure indices (ABI) were found to be significant higher than STP and toe/brachial indices (TBI) both at the start of the study and after six years. At start: 157 ± 41 vs 117 ± 33 mmHg, p <0,0001. At follow up: 156 ± 46 vs 106 ± 33 mmHg, p <0,0001. Index: At start: 1,00 ± 0,24 v s 0,77 ± 0,21, p <0,0001. At follow up: 1,04 ± 0,26 vs 0,71 ± 0,22 p <0,0001. TSP and TBI were significant reduced after six years: p< 0,0001 and p< 0,05 respectively. At the start of the study nine extremities (8 patients) were found with a ADP ≤ 80 mmHg and twenty-five extremities (17 patients) with a STP≤ 80 mmHg. Among these patients with low ADP at the start 2 of 8 died during follow up. Among the patients with low TSP at start 8 of 17 died during follow up. All patients (n= 4) with an initial TSP ≤ 50 mmHg died during follow up. After six years the Doppler examination found six extremities and the toe pressure method thirty-three extremities with a systolic pressure ≤ 80 mmHg. The 19 patients who died of cardiovascular diseases had significant lower TSP at the start of the study (98 ± 40 vs 117 ± 33 mmHg; p< 0,05), however they were also significantly older (73 ± 7 vs 65 ± 11 years; p< 0,001).The pulse oximetric method gave significantly more pathological indices at the end of the study. (Doppler index ≤ 0,8, compared with pulse oximetric index ≤0,6), Doppler: 32/173; pulse oximetric method 58/173; p=0,003. However, the Doppler method gave significantly more indices above 1,3 compared with the pulse oximetric method (28/173 vs 0/173, p= 0,003).
Conclusions: This study demonstrates that ankle Doppler pressure measurements overestimates peripheral pressure in a typical primary health care diabetes population and is not suited for epidemiological studies. In diabetic patients toe systolic pressure is reduced during ageing and can be seen as a marker for general arteriosclerosis disease. In the screening situation, this pulse oximetric toe pressure method seems to be valuable since it can be performed in outpatient clinics and handle large number of patients in a short time and avoid the problem of media sclerosis.
Support: Praktikertjänst, Sweden

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Morbidity and mortality among diabetic patients hospitalized with foot ulcers, Dar es Salaam, Tanzania

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Background and Aims: To characterize the epidemiology and outcome of foot ulceration among diabetes patients hospitalized at Muhimbili National Hospital (MNH), Dar es Salaam, Tanzania.
Materials and Methods: A foot ulcer case was defined as any adult diabetes patient who was admitted to the MNH inpatient medical service with an ulcer at or below the ankle joint during January 1997 – September 2004 (study period). Following hospital admission and informed consent, detailed clinical and epidemiologic data were recorded for each patient. Presence of microvascular (i.e., retinopathy, nephropathy, or both) and macrovascular (i.e., one or more of peripheral vascular disease [PVD], cerebrovascular disease, or ischaemic heart disease) disease were documented. In addition, clinical progression, management details and outcome (healed, healing in progress, discharge from hospital, or death).
Results: Of 2786 diabetic patients admitted to the MNH inpatient service during the study period, 454 (16.3%) met the case definition. Of these 454 patients, 310 (68.3%) were male, 438 (96.5%) were of African ethnicity (vs. 3% Asian), 415 (91%) had type 2 diabetes, 369 (81%) had peripheral neuropathy (PN), 138 (31%) had peripheral vascular disease (PVD), 122 (27%) had neuro-ischaemia, and 208 (46%) had microvascular disease. Patient characteristics were as follows: median age: 54 (range: 21–96) years; median duration of diabetes: 5.0 years (range: 1 week-30 years). At presentation, the median duration of ulcer was 3 weeks (range: 2 day–38 weeks) and the ulcers in 255 (56%) study-patients had progressed to gangrene (Wagner ≥4); 199 (44%) patients subsequently underwent amputation and 119 (26.2%) patients died. Factors associated with increased mortality included macrovascular disease (relative risk [RR]: 2.0; 95% confidence interval [CI]: 1.5–2.8, p <0.0001), neuro-ischaemia (RR: 1.9; CI: 1.42.6, p <0.0001), ulcers of duration >3 weeks (RR: 1.4; CI: 1.0–2.0, p<0.01), or Wagner score ≥4 (RR: 3.0; CI: 2.0–4.5, p <0.01). Patients with gangrenous ulcers (Wagner score ≥4), who did not undergo surgery, were significantly more likely to die compared with patients who underwent surgery and limb amputation (RR: 2.5; CI: 1.9–3.4, p <0.0001).
Conclusion: Foot ulcers were associated with significant morbidity and mortality among diabetes patients admitted to the MNH inpatient, acute medical service in Dar es Salaam. Mortality rates were highest in those patients with large vessel disease or among patients with gangrenous ulcers that was not treated surgically. Preventive efforts should focus on educating diabetes patients to present to hospital at the earliest onset of foot lesions before the onset of gangrene.

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MRSA, is it an underestimated predictor of survival in diabetic foot ulcers?

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Background and Aims: The present study aims to examine the long term outcome in terms of mortality of type 2 diabetic patients hospitalized primarily due to infected foot ulcers.
Materials and Methods: A total of 75 consecutive type 2 diabetic patients who were hospitalized with newly diagnosed foot ulcer from 1997 to 2002 were included. The mean age was 58 ± 10.5 years, the mean duration of diabetes was 13.8 ± 7.7 years, and 64% of patients were male. Survival rates were determined by telephone contact with 62 available patients and family members. Mean duration of follow up was 48 ± 25 months. Age, diabetes duration, amputation, HbA_{1c}, inflammatory parameters (C-reactive protein [CRP], white blood cell count [WBC], erythrocytes sedimentation rate [ESR]), ulcer grade according to Wagner classification, wound culture, co-existing albuminuria, ischemic heart disease (IHD) and peripheral vascular disease were the factors examined. We used univariate correlation and logistic regression analyses to examine the independent effect of each variable on mortality rate. Kaplan-Meier survival curves were generated and the log-rank test was used to test the equality of survival functions between the groups.
Results: The mean HbA_{1c} was 8.8 ± 1.8. In 44 patients (58.7%) foot ulcer was related to neuropathy solely, while 31 patients (41.3%) had neuroischemic ulcer. IHD was diagnosed in 44 patients (48%). Albuminuria was detected in 29 patients (38,6%). Number of patients having Wagner grade 3 or more severe foot ulcer associated with severe soft tissue infection and osteomyelitis, was 39 (52%). The predominant pathogenic microorganism, methicillin-resistant Staphylococcus auereus (MRSA), was isolated in 24 patients (32%). Grafting or minor amputation was performed in 27 patients (36%) and major amputation in 11 patients (14.7%). The mortality rate was 34% in 62 cases. Cardiovascular (CVD) disease was the major cause of death in these patients. Mortality rates of patients having IHD, leukocytosis (WBC>10.000/mm^3; p=0.019) and positive MRSA swab culture were significantly higher than patients without these characteristics (50 vs. 18.7%, p=0.01; 44.4 vs. 25.7%, p=0.03 and 50 vs. 19.4%, p= 0.006, respectively). Co-existing IHD (p=0.013), isolation of MRSA (p=0.030) and leukocytosis were the independent variables increasing the mortality rate.
Conclusion: In this prospective study, we determined that co-existing IHD, presence of MRSA infection and leukocytosis were the most significant fac-

tors that affected mortality rate in patients with diabetic foot ulcer. MRSA is known to be the most common pathogen in diabetic foot ulcers and associated with a prolonged healing time. We, therefore hypothesize that MRSA results in a more severe and more complicated inflammatory response than other pathogens and significant correlation with leukocytosis seems to support our hypothesis. Many studies revealed the important role of inflammation in IHD. In diabetic patients with foot ulcers, increased mortality due to IHD may be attributable to excessive inflammation triggered by MRSA infection, partially.

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Asymptomatic onychomycosis is more common in high risk diabetic feet rather than in subjects with raised HbA_{1c}

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Background and Aims: It is believed that poor glycaemic control predisposes to fungal infection, however it is not clear if onychomycosis is common with raised HbA1c in diabetic subjects. The aim of this study was to find out the prevalence and risk factors of onychomycosis in asymptomatic diabetic subjects.
Materials and Methods: Subjects attending community podiatry clinic were studied by collecting nail clippings during routine feet screening. Nail clippings were digested in 20% KOH prior to microscopy. Clippings were cultured on Saboraud's agar with chloramphenicol.
Results: 88 consecutive patients with diabetes [mean age 78.4 (SD 10.2) and duration 7.9 (SD 8.1) years] were studied over 2 years. Onychomycosis, defined by presence of fungus on microscopy or growth of typical dermatophytes on culture, was present in 28.4% subjects. There were no differences in age, duration of diabetes and HbA1c between subjects with and without onychomycosis. However, there was significantly ($p<0.05$) higher prevalence of absent foot pulses (12.7% vs 16%), absent 10 gram mono-filament sensation (22.2% vs 28%), foot deformity (69.4% vs 84%) and history of foot ulcers (3.2% vs 8%) in subjects with onychomycosis. There was no difference in lipid profile, serum creatinine and microalbuminuria between two groups.
Conclusion: We found the risk factors for onychomycosis to be similar to that of foot ulcers and further studies are needed to clarify its role in the pathogenesis and severity of foot ulceration.
Support: Seedcorn Fund

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Peripheral and autonomic neuropathy: assessment and prognosis

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Usefullness of the indicator plaster neuropad for the diagnosis of peripheral and autonomic neuropathy in patients with diabetes mellitus

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Background and Aims: The indicator plaster neuropad (IPN) is based on the color change of a cobalt II compound (placed on a commercially available sticker) from blue to pink, after exposure to dermal foot perspiration. Lack of perspiration (which results in non-change of the neuropad sticker color), is considered as a sign of peripheral neuropathy (PN) which in turn is a major risk factor for the development of diabetic foot syndrome. Perspiration is autonomic nervous system dependent. Autonomic neuropathy (AN) is relatively common in diabetic patients and lack of perspiration is often one of its clinical features. Aim of this study was to evaluate the sensitivity and specificity of IPN for the detection of sensory PN and cardiac AN, in patients with diabetes mellitus (DM).
Materials and Methods: The study population consisted of 116 patients (64 men and 52 women, mean age 61.6 years) with DM (9 with type 1 and 107 with type 2 diabetes) of at least 5 years duration, randomly recruited from the diabetologic outpatient clinic of our hospital. IPN was placed at the plantar surface of the first metatarsal of both feet. Patients were examined for PN by using a the neuropathy symptoms score (NSS), the neuropathy disability score (NDS) and the vibration sensitivity threshold. Cardiac AN was examined by using the classical battery of the Ewing tests.
Results: PN was documented in 50 out of 116 patients (43.1%). The sensitivity of IPN in diagnosing PN was found 86% (43/50 patients) while its specificity was 68.2% (45/66 patients). Positive predictive value was 67.2% (43/64 patients) and negative predictive value was 86.5% (45/52 patients). Cardiac AN was documented in 43 out of 112 patients (38.4%). The sensitivity of IPN in diagnosing cardiac AN was found 58.1% (25/43 patients) and its specificity was 44.9% (31/69 patients). The sensitivity of IPN in detecting those patients with combined PN and cardiac AN was 80.7% (21/26 patients).
Conclusion: IPN has a high sensitivity and a rather low specificity for the detection of PN in patients with diabetes mellitus, while both sensitivity and specificity concerning the detection of cardiac AN by this system are low. This finding along with the simplicity of the technique, suggests that IPN may be a useful screening tool for PN in patients with diabetes mellitus.

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Assessment of diabetic autonomic neuropathy in type 2 diabetic patients using neuropad: A new indicator plaster for detection of disturbed sweat secretion

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Background and Aims: The presence of autonomic dysfunction in diabetic patients predicts a poor prognosis. Clinical tests are limited to heart rate variability and blood pressure measurement. Moreover, it is difficult and needs long time to carry out. Autonomic sudomotor neuropathy is associated with reduction of plantar sweating. Early diagnosis of the sudomotor component of autonomic neuropathy may be helpful to detect diabetic autonomic dysfunction. Therefore, the aim of this study was to evaluate whether the new indicator plaster (Neuropad) was suitable screening test for diabetic autonomic neuropathy.
Materials and Methods: This study included 185 type 2 diabetic patients (78 men and 107 women) with a mean age of 57.4 ± 10.2 years. The average duration of the diabetes was 8.5 ± 5.0 years (median 5 years). The control group comprised 19 healthy young volunteers (< 30 yrs old). We carried out four autonomic function tests (E/I ratio, Valsalva, 30:15 ratio, Orthostatic-BP) as conventional standard tests. Indicator plasters were applied to both soles of patients. Autonomic neuropathy was assessed by means of color change in the indicator plasters (normal response: full color change within 10 minutes). And then we compared the results of both tests.

Results: Autonomic neuropathy was diagnosed in 163 patients (79.9%) with conventional tests and 137 patients (67.2%) were positive with indicator plaster. Color change of the plaster in the right sole was associated with color change in the left sole (p=0.0001). We calculated kappa value to estimate the agreement between Neuropad and conventional tests for DAN. The weighted kappa value was 0.38. The sensitivity of the indicator plaster for diagnosis of autonomic neuropathy was 76.7% and specificity was 70.7%. In the logistic regression analysis, the following parameters; duration of diabetes, sex, HbA1c, serum total cholesterol and blood pressure were not significant factors for Neuropad results, whereas the age of patients could influence.
Conclusion: These results suggest that the indicator plaster is suitable of use in the screening test for diabetic autonomic neuropathy.

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The steel-globe: a new test for the diagnosis of diabetic peripheral neuropathy and the diabetic foot
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Background and aims: Foot lesions still remain a major cause of morbidity among patients with Diabetes Mellitus. Neuropathy is recognized as one of the most important risk factors for diabetic foot lesions. Thus, the aim of the present study was to evaluate a new diagnostic test, the steel-globe, in the diagnosis of diabetic neuropathy and in the detection of the diabetic foot among neuropathic patients.
Materials and methods: This study enrolled 109 diabetic patients (65 men, 87 type 2 diabetes). These were divided into Group A (39 patients, 30 men, mean age 61.5 ± 9.8 years, mean diabetes duration 17.9 ± 3.1 years, with peripheral neuropathy and diabetic foot), Group B (36 patients, 18 men, mean age 63.7 ± 10.1 years, mean diabetes duration 17.6 ± 2.9 years, with peripheral neuropathy without diabetic foot) and Group C (34 patients, 17 men, mean age 52.1 ± 10.4 years, mean diabetes duration 9 ± 1.1 years, without neuropathy and without diabetic foot). A control group D of 21 healthy volunteers (9 men, mean age 46.7 ± 8.7 years) was also included. Diabetic neuropathy was assessed with the aid of the standardized tuning fork (128 Hz), the 10 g Semmes-Weinstein Monofilament, the Vibration Perception Threshold and the thermal perception threshold. Neuropathy was diagnosed when at least two of these tests were abnormal. Diabetic foot was defined as history of foot ulcer or evidence of Charcot osteoarthropathy. Patients were examined with a special steel-globe (its diameter varying between 1.5 and 4 mm) stuck to a plaster, which was applied in the plantar area over the second metatarsal head of each foot successively, while an empty control plaster was applied on the contralateral foot. The smallest diameter of the steel-globe that was felt as foreign body was recorded.
Results: Mean diameter of the steel-globe that was felt as foreign body was 3.63 ± 0.54 mm in Group A, 2.5 ± 0.69 mm in Group B, 1.91 ± 0.58 mm in Group C and 1.65 ± 0.15 mm in Group D (all differences between the groups were highly significant at p<0.001). A significant correlation was observed between the steel-globe and the monofilament (r=0.561, p=0.001), the Vibration Perception Threshold (r=0.47, p=0.001) and the thermal perception threshold (r=0.59, p=0.001). Use of a steel-globe with a diameter of 2 mm had a sensitivity of 84% and a specificity of 100% for the diagnosis of neuropathy. Use of a steel-globe with a diameter of 3 mm had a sensitivity of 84.6% and a specificity of 86.1% for the diagnosis of the diabetic foot among patients with neuropathy.
Conclusions: The new steel-globe appears to have a high sensitivity and specificity for the diagnosis of peripheral neuropathy. The new diagnostic test also has a high sensitivity and specificity for detection of the diabetic foot among neuropathic patients.

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Sympathetic denervation of the heart closely associated with disturbed heart rate variation during deep breathing amongst type 1 diabetic patients
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Background and Aims: I-123-MIBG scintigraphy visualises the sympathetic nerve distribution and density in vivo. The aim of this study was to relate putative sympathetic denervation of the heart to cardiac autonomic neuropathy (CAN) as demonstrated by conventional CAN tests.
Materials and Methods: I-123-MIBG scintigraphy was conducted in 29 type 1 diabetic patients. The patients had no previous history of coronary ischemia and showed normal coronary perfusion according to myocardial scintigraphy. SPECT images covering different regions over the heart were obtained and myocardial MIBG uptake/perfusion ratios were calculated. Based on planar images, a heart to mediastinum ratio (H/M ratio) was also calculated. CAN was assessed by the heart rate reaction to deep breathing (E/I ratio) and to tilt (acceleration [AI] and brake index [BI]).
Results: 11 patients showed abnormal E/I ratios, 11 abnormal AI, and 1 patient an abnormal BI. Patients with an abnormal E/I ratio showed a significantly lower median H/M ratio than patients with a normal E/I ratio (1.67 [0.21] vs. 1.92 [0.36]; P=0.001) (median [interquartile range]). However, patients with an abnormal E/I ratio showed a significantly higher median MIBG-uptake/perfusion ratio in the septal region of the myocardium than patients with a normal E/I (1.09 [0.09] vs. 1.02 [0.12]; P=0.039). Indeed, patients with abnormal AI also showed a significantly higher median MIBG-uptake/perfusion ratio in the septum than patients with normal AI (1.1 [0.08] vs. 1.02 [0.12]; P=0.003).
Conclusions/Interpretation: CAN, detected by conventional tests, was associated with sympathetic denervation of the myocardium. Patients with an abnormal E/I ratio showed a general sympathetic denervation of the heart (low H/M ratio). Low E/I ratio and low AI reflect disturbed parasympathetic nerve function and were here associated with increased sympathetic nerve activity in the septum of the heart. This suggests that proximal sympathetic nerves were preserved in the heart and/or that parasympathetic denervation increases the sympathetic receptor binding in this region. Increased septal sympathetic nerve activity among patients with parasympathetic neuropathy may increase the risk for cardiac arrhythmias.
Support: Swedish Diabetes Association/Swedish HeartLung Foundation

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Usefulness of ambulatory blood pressure monitoring in predicting the presence of autonomic neuropathy in type 1 diabetic patients
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Background and Aims: Although the prognostic meaning of the reduced nocturnal fall of blood pressure (BP) (nondipping) is well known in diabetic patients, its predictive value with regard to the presence of autonomic neuropathy (AN) is not established. Moreover, a nocturnal decline in the daytime BP by 0% or less, has been proposed as a more adequate threshold in defining nondipping in alternative to the previous 10% criterion. We investigated whether nondipping could be assumed as a diagnostic index for AN, and assessed its accuracy in discriminating between type 1 diabetic patients with and without AN.
Materials and Methods: In 87 type 1 diabetic patients with normal renal function (age 36 ± 11, duration 17 ± 9 years, BMI 24 ± 4 Kg/m^2, HbA1c 7.9 ± 1.7%, serum creatinine 0.76 ± 0.18 mg/dl, 24 h albumin excretion rate 33 ± 75 μg/min, casual BP 119/73 ± 14/9 mmHg, 23 with microalbuminuria, 12 with hypertension, 40 men), we performed 4 standard cardiovascular tests and 24 h BP monitoring, and calculated the percentage day-night change (Δ) in systolic (sBP) and diastolic BP (dBP).
Results: According to cardiovascular tests, 18 patients had early AN (≥ 1 abnormal test results), 32 definite AN (≥ 2 abnormal test results), and 37 normal tests. 16 patients had Δ sBP and or Δ dBP ≤0%, and 13 out of them exhibited definite AN (Chi^2=10.2, p=0.001). In a multiple logistic regression with adjustment for sex, age, and BMI, the odds ratio for having AN was 7 times higher in patients with Δ BP ≤0% as opposed to those without (Odds Ratio 6.97, 95% C.I. 1.4-34.9, p=0.018). Using Receiver Operating Characteristic (ROC) analysis, Δ BP showed an acceptable accuracy in discriminating between patients with and without AN (area under the ROC curve 0.68 ± 0.06 (95% C.I. 0.56–0.78) for both Δ sBP and Δ dBP). Adequate cutoff values seemed to be 0% for Δ sBP [sensitivity 26%, specificity 95%, positive predictive value (PPV) 87% (95% C.I. 70–104), negative predictive value (NPV) 49% (95% C.I. 37–60)], and 5% for Δ dBP [sensitivity 26%, specificity 92%, PPV 81% (95% C.I. 62–100), NPV 48% (95% C.I. 36–59)], endowed with the highest values of Likelihood Ratio (LR) (5.2 and 3.3, respectively).

Diagnostic characteristics of different cutoff values for Δ BP

Δ sBP 0%	26%	95%	87% (70–104)	49% (37–60)	5.2
Δ sBP 5%	42%	86%	81% (66–96)	52% (39–64)	3
Δ sBP 10%	70%	51%	66% (48–84)	56% (43–68)	1.4
Δ dBP 0%	14%	95%	78% (51–104)	45% (34–56)	2.8
Δ dBP 5%	26%	92%	81% (62–100)	48% (36–59)	3.3
Δ dBP 10%	40%	78%	71% (54–88)	49% (36–62)	1.8
Cutoffs of Δ BP	Sensitivity	Specificity	PPV (C.I.)	NPV (C.I.)	LR

Conclusion: In type 1 diabetic patients with normal renal function, a value of Δ BP ≤0%, that corresponds with a nighttime BP equal to or higher than the daytime BP, identifies the presence of AN with a very high chance. Day-night change in BP could be considered an adjunctive marker of autonomic dysfunction provided with a high specificity and low sensitivity.

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Orthostatic cardiac decompensation may be the cause of increased clinical autonomic neuropathy in females

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Background and Aims: Symptomatic autonomic neuropathy (AN) in type 1 diabetic subjects is believed to be more common in females and is associated with serious adverse outcomes. However, most studies that have looked at sub-clinical AN do not show any gender differences in the various measures of cardiac autonomic function tests (CAFT). The Sheffield Prospective Study sought to investigate if gender differences were related to the development of abnormal CAFT.
Materials and Methods: 66 newly diagnosed type 1 diabetic patients [mean age 31 ± 9(SD) duration (3 years± 2); 41 males & 25 females] were identified and followed for 9 years. They had detailed neurological assessment (symptoms and signs score, nerve conduction, qualitative sensory tests and CAFT) and blood samples taken for detailed biochemical and haemorrheological analysis. CAFT was done as per O'Brien protocol and involved measurements of R-R variation at rest, on standing, on deep inspiration and with Valsalva manouvre along with measurement of orthostatic drop of systolic blood pressure. All investigations were performed at baseline, 3 years and 9 years. AN was defined as abnormalities in 2 or more measures of CAFT.
Results: At the 9 years follow up, 51 patients (33 males) were studied and a significant number of females (16.7% of females and 9.1% of males; p=0.03) developed AN. There were no gender differences in age, duration of diabetes and HbA_{1c}. On detailed analysis of the various components of CAFT there was a significant deterioration in the mean annual rate of orthostatic blood pressure in females (0.68 mm Hg vs 0 mmHg per year; p =0.003). On the other hand there was an improvement (p = 0.01) in the annual rate of change in R-R variation on standing, but no difference (p>0.05) during rest, deep inspiration or Valsalva manoeuvre.
Conclusion: This prospective study shows that cardiac AN is more common in females primarily as a result of orthostatic hypotension despite a modest but significant improvement in R-R variation on standing.
Support: Diabetes UK

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In asymptomatic diabetic patients the poor prognosis related to artery disease is aggravated by cardiac autonomic neuropathy

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Background and Aims: Cardiac autonomic neuropathy (CAN) is associated with an impaired cardiac prognosis. We have previously shown that after a mean 4.5-year follow-up cardiac prognosis was even poorer in the diabetic patients with both CAN and silent myocardial ischemia (SMI). The aim of this study was to determine in the same cohort whether CAN may aggravate the prognosis related to artery disease over a longer follow-up.
Materials and Methods: One-hundred and twenty patients, 63 men and 57 women, aged 55 ± 9 years, diabetes duration 16 ± 8 years, with no cardiac history and with a normal resting ECG at baseline, were included. CAN was defined by at least one out of three abnormal heart rate variation tests (deep-breathing, lying-to-standing, Valsalva), SMI by an abnormal thallium myocardial scintiscan, and peripheral occlusive artery disease (POAD) by stenoses > 50% on lower limb ultrasound examination.
Results: Eleven patients were lost to follow-up. The remaining 109 patients were followed during 7.9 ± 3.3 years. A major cardiac event (death, myocardial infarction, coronary revascularization, heart failure) occurred in 26 patients. POAD (OR 3.4 [95% CI 1.3–8.8]; p = 0.013), SMI (OR 4.0 [1.7–9.4]; p = 0.002), and diabetic retinopathy (OR 4.0 [1.3–6.4]; p = 0.012) were significant predictors of events. When separating patients according to the presence or absence of CAN, POAD (OR 6.1 [1.3–27.4]; p = 0.019) and SMI (OR 17.4 [1.8–168.8], p = 0.014) were predictive of events only in patients with CAN.
Conclusion: This study suggests that in diabetic patients CAN does not remain a significant predictor of events over a longer follow-up but seems to aggravate the prognosis related to artery disease.

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A link between endocrine testis function and autonomic activity in patients with diabetes and erectile dysfunction?

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Background and Aims: Primary hypogonadism is uncommon in patients with erectile dysfunction (1–2% of affected). However, we observed that total testosterone value was in the lower <2.5° percentile in 32% of diabetics with erectile dysfunction. Recent evidences show that testosterone enhances Nitric Oxide (NO) and parasympathetic central nervous activity. The aim of this study was to verify a possible link between testosterone plasma levels and autonomic function evaluated by cardiovascular tests.
Materials and Methods: 544 type 2 diabetic patients, aged 56 ± 9 years (mean±SD), with duration of disease 8 ± 4 years and acceptable metabolic control (HbA1c = 7.4 ± 0.6), were studied. They were divided in two groups: patients without (group 1) and with (group 2) autonomic dysfunction, on the basis of their Heart Rate response to Deep Breathing with a cut off point of <1.15 or > 1.15 EI respectively. Lying to Standing (LS) Heart Rate variations were also analyzed and here reported. Testosterone was evaluated by RIA method.
Results: The table shows the parameters considered of the two groups of diabetic patients examined. As for testosterone values we found a significant difference between patients with and without autonomic dysfunction (t=3.9; p<0.01). Moreover, a linear correlation was observed between LS (a test which evaluates parasympathetic function, as well as the central parasympathetic outflow), and testosterone values (p<0.03; r= 0.45).
Conclusion: These data concern a large cohort of patients with diabetes and erectile dysfunction and show a possible link between autonomic impairment and a lower endocrine testis activity. We need further studies to verify hypothesis that an endocrine therapy with testosterone could increase parasympathetic function in patients with diabetes and erectile dysfunction.

Table: Parameters of diabetic patients

	EI	n	Mean	SD
Age (years)Group 1	> 1.15	369	57.5	7.5
Age (years) Group 2	< 1.15	175	58.6	9.6
LS Group 1	> 1.15	369	1.18	0.15
LS Group 2	< 1.15	175	1.05	0.06
Testosterone (nmol) Group 1	> 1.15	369	28.52	7.2
Testosterone (nmol) Group 2	< 1.15	175	16.21	6.0

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Relationship between diabetic gastroparesis and endothelin receptors in the streptozotocin-induced diabetic rats

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Background and Aims: Diabetic gastroparesis is suggested to result from not only autonomic neuropathy but also the disorder of spontaneous rhythmic motility of gastric smooth muscle. Recent studies have revealed that function of intracellular Ca2+ stores may play an important role in the generation of spontaneous contraction. Endothelin-1 (ET-1) is known to facilitate Ca2+ release from intracellular stores. However, little is known

about the effects of ET-1 on diabetic gastroparesis. Attempts were made to investigate the difference of the effects of ET-1 between normal rats and storeptozotocin (STZ)-induced diabetic rats and to clarify the relationship between diabetic gastroparesis and ET receptors.
Materials and Methods: STZ-induced diabetic rats were made by the intraperitoneal injection of STZ (60 mg/kg body weight) on SD rats (6 weeks old) and bred for a month. Age-matched SD rats were used as normal rats. Isometric mechanical recording techniques were used to measure spontaneous contraction of a strip (2 × 10 mm) of smooth muscle from gastric antrum.
Results: In normal rats (n=22), the average resting tension was 0.16 ± 0.01 g and the frequency and amplitude of spontaneous contraction was 2.6 ± 0.1/min and 0.16 ± 0.01 g respectively. In diabetic rats (n=12), these three parameters were not altered significantly. ET-1 (10 nM) significantly increased the resting tension (control: 0.17 ± 0.02 g; ET-1: 0.83 ± 0.08 g, n=11, P<0.05) and the frequency (control: 2.6 ± 0.1/min; ET-1: 2.9 ± 0.1/min, P<0.05), but not altered the amplitude. In the diabetic rats, ET-1 increased only the resting tension (control: 0.19 ± 0.02 g; ET-1: 0.97 ± 0.10 g, n=6, P<0.05). Interestingly, sarafotoxin S6 c (S6 c, 10 nM), a selective ET type B (ETB) receptor agonist, increased all three parameters, naming the resting tension, the frequency and the amplitude of spontaneous contraction, in the normal rats. However, S6 c increased only the resting tension (control: 0.18 ± 0.03 g; S6 c: 1.83 ± 0.20 g, n=5, P<0.05) in the diabetic rats. To investigate the agonistic action of ET-1 on ETA receptors, ET-1 (10 nM) was applied in the presence of BQ788 (1 mM), a selective ETB receptor antagonist. In the normal rats (n=5), ET-1 significantly increased the resting tension (control: 0.19 ± 0.03 g; BQ788 1.01 ± 0.14 g, P<0.05), decreased the amplitude (control: 0.56 ± 0.05 g; BQ788 0.38 ± 0.04 g, P<0.05), however did not alter the frequency in the presence of BQ788. In the diabetic rats (n=5), ET-1 increased the resting tension (control: 0.13 ± 0.02 g; BQ788 0.74 ± 0.07 g, P<0.05) and the amplitude (control: 0.33 ± 0.03 g; BQ788 0.82 ± 0.08 g, P<0.05) in the presence of BQ788.
Conclusion: These results indicate that ET-1 facilitates the frequency of spontaneous contraction through ETB receptors, and elevated the resting tension through ETA receptors in the normal rat gastric antrum. However, the selective ETB receptor agonist did not facilitate the frequency in the diabetic rats. It is concluded that diabetic gastroparesis is associated with the disorder of ETB receptors signal transduction.

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Subclinical polyneuropathy in patients with diabetes mellitus type 1 as the earliest chronic complication
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Background: Distal symmetric polyneuropathy (PNP) is the most common form of diabetic neuropathy and supposed to be primary a disorder of sensory nerves.
Aim: To verify that PNP in patients with diabetes mellitus type 1 (DM1) starts soon after onset of disease and not after five years as stated in literature.
Materials and Methods: We performed study in patients with DM1, in which duration of disease ≤5 yrs and age of the patients ≤40 yrs were inclusion criteria. Neurologic damage of the peripheral nerves was estimated clinical by neurologist, by biothesiometry and quantitative autonomic testing (QAT). Nerve conduction velocity (NCV) and amplitude (AMP) of motoric and sensitive nerves (tibial, peroneal, plantar medial, and sural) were evaluated by electromyography (EMG). Risk factors of PNP (age, height, levels of serum lipids, hypertension, smoking, retinopathy) were accounted as well as parameters of compensation (HbA1c, history of ketoacidosis in last year) were assessed.
Results: NCV and AMP of tibial, peroneal and sural nerve were in normal range. Overall 38 (62,3%) patients had decreased NCV of the plantar medial nerve 32,6 ± 7,6 ms and thus fullfilled criteria for subclinical stage of PNP. For further analysis, patients were divided into two groups according to the duration of DM1. Group 1 consisted of 30 patients with duration of DM1 <12 months (3,6 ± 2,9), group 2 comprised 31 patients in which DM lasted ≥12 months (31,0 ± 14,5). Average value of NCV of the plantar medial nerve was 31,3 ± 7,6 in group 1, and 33,8 ± 7,6 in group 2, respectively (P>0.05).
Conclusion: Our results show that subclinical form of PNP is present earlier than generally accepted 5 years after onset of DM1. It seems, the plantar medial nerve is first to be affected as assessed by the EMG studies. Poor glycemic control is the major risk factor of PNP. Probably some other and not clearly understood factors contribute to PNP.

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Prevalence of diabetic foot syndrome in Germany 2002–2004
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Aim: Reports on the prevalence of the diabetic foot syndrome are often overestimated due to the bias caused by collecting the data from non population based samples in hospitals or ambulatory diabetes clinics. In a special survey (Diabetes-TÜV) by a health insurance the prevalence was assessed on the primary care level.
Method: A special foot documentation of 4869 patients (age 65.2 years; 44% women, 7.5% type 1, diabetes duration 8.8 years; HbA1c DCCT adjusted 6.64%; BMI 29.7 kg/m^2; BP 142/81 mmHg) insured by the Deutsche Betriebskrankenkasse was carried out in 351 German practices from 2002–2004. The results of the foot examination were recorded in a graph for the left and the right foot separately. The signature of the patients on the documentation was a prerequisite for the remuneration of physicians. The patients received a copy of the results. Physicians received a special payment for each complete and signed documentation.
Results: Only 1% of the patients had incomplete results. Actual ulcus/gangraene was present in 43 patients (0.9%), previous ulcus/gangraene in 46 patients (0.9%), lower limb amputation in 75 patients (1.5%). Altogether a diabetic foot syndrome (actual or previous ulcus, gangraene or amputation at lower limb) was present in 141 patients (2.9%). Amputation level: toes 46%, forefoot 8%, below knee 17%, above knee 22%, others 8%. Polyneuropathy (Semmes-Weinstein-Monofilament negative at planta pedis) was diagnosed in 9.8%, A. dorsalis pedis was not palpable in 12.5%; A. tibialis posterior was not palpable in 14.9%; Callus was present in 35%, skin mykosis in 7.8%, nail mykosis in 34.7% and unguis incarnatus in 7.7%.
Conclusion: The completed documentation countersigned by the patients was of an excellent quality. The prevalence of actual ulcera or gangrene was lower and the amputation rate was comparable to the results of other larger surveys in Europe (Komar UK 1994; Abbot UK 2002, Altenhofen D 2002).

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Diabetic peripheral neuropathy in Germany: relative prevalence, disease characteristics and health related quality of life for pre-defined severity stages

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Background and Aims: Diabetic Peripheral Neuropathy (DPN) is a risk factor for ulcers and amputations of the lower extremities. Management of DPN focuses on prevention by optimal blood glucose level maintenance and on foot care and education, and treating symptoms and complications such as ulcers. In the framework of the DIMICO (DIabetes MIcrovascular COmplications) study, the analyses presented investigated epidemiological, medical and Health Related Quality of Life (HRQL) aspects of DPN by predefined severity stages
Materials and Methods: A cross-sectional epidemiological study (N=1480; type 1 diabetes: N=408, type 2 diabetes: N=1072) evaluated the relative prevalence of DPN severity stages. In this observational study, medical characteristics of patients with DPN were determined for each stage of disease severity using a stratified sampling design (year 2002; N=185). Patients completed generic (SF-12©) and disease-specific (QOL-DN©) HRQL instruments
Results: Among type 1 and 2 diabetes patients with DPN, prevalence of predefined severity stages was as follows: sensory-motor DPN without symptoms (32%) and with symptoms (47%) were most frequently reported, 11% of DPN patients suffered from neuropathic foot/ulcer or deformities, 10% had lower extremity amputation in 2000 (4%) or anytime before 2000 (6%). Among patients with DPN, 93% had concomitant diseases: hypertension (81%), hypercholesterolemia (49%), coronary artery disease (38%) and hyperlipidemia (37%). Other microvascular complications were reported (retinopathy: 28%; nephropathy: 25%). In more severe stages of DPN, combinations of DPN symptoms with other forms of neuropathy were more frequent; e. g. 25% of amputations were associated with such other forms of diabetic neuropathy.
On the generic SF-12 physical component HRQL scale, a mean reduction in score from 43.2 for patients suffering from sensory motor neuropathy without symptoms to 35.2 for sensory motor neuropathy with symptoms and to 32.4 for patients with amputation before the year 2002 was observed. The SF-12 mental component scores were affected less by DPN severity: patients with sensory motor neuropathy without symptoms were in the normal range (51.9), patients with sensory neuropathy with symptoms (44.3) and amputation before 2002 (45.1) or in 2002 (43.5) showed lower mental HRQL. Likewise, typical signs and symptoms of diabetic neuropathy and activities of daily living were worse with increasing DPN severity as measured by QOL-DN, a disease specific HRQL instrument: the total score increased (indicating worsening of HRQL) from 24.8 for sensory neuropathy without symptoms to 41.7 for sensory neuropathy with symptoms and finally to 60.4 for patients with amputation before 2002. Interestingly, patients with more severe stages were not found to exhibit higher blood glucose or HBA1c levels during the documentation period.
Conclusion: DPN progression was associated with higher comorbidity rates and lower generic and disease specific physical HRQL. Mental HRQL was less affected in comparison. This demonstrates that special emphasis should be placed on avoiding the progression of DPN, as more sever stages of DPN are associated with lower HRQL and higher risk of ulcers and amputations.
The study has been sponsored by Eli Lilly and Company

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The disease burden of diabetes mellitus foot syndrome in Nigerians

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Background and Aims: The major part of the burden of people with diabetes mellitus (DM) is their reduced quantity and quality of life. This is due to the acute and chronic complications of which diabetes mellitus foot syndrome (DMFS) takes the greatest toll. In many countries, DMFS is the most common cause of hospitalization among people with DM. This DM complication is a significant economic problem since it often results in prolonged hospitalizations and sometimes amputation.
Most studies on the disease burden of diabetes foot ulceration were carried out in developed countries and till date, no indigenous study has addressed the burden of foot ulceration in Nigerians with DM. This study attempts to determine the disease burden of this important DM complication. The results are hoped will be of benefit not only to diabetologists but also to sensitize people with DM and policy makers on the scope of the problem associated with DMFS.
Materials and Methods: The study was carried out at the Lagos University Teaching Hospital (LUTH), Lagos, Nigeria. The working definition of disease burden encompassed prevalence, morbidity, mortality and the direct economic costs of DMFS.
The estimates of prevalence-the "Capture-Recapture" and intensive case counting methods were used-, mortality and morbidity were determined from records of admissions and associated deaths over a six- year period (1995–2000).
The direct economic costs of foot ulceration were derived from the costs incurred from In-patient days, tests, drugs/medications, surgery and other miscellaneous units of services.
Data were analyzed using the Statistical package for social sciences-SPSS and Epi info version 6.4. The test statistics used included Student's t test, Chi squared test and correlation.
Results: The total number of people with DM seen in LUTH from 1995–2000 was 1,500, the hospital prevalence of DMFS using the Capture-Recapture method being 9.5%.
A total of 7,253 medical admissions were made in this 6-year period and of this number, 827(11.4%) were DM related. DMFS related admissions were 97 in number and this made up 1.3% and 11.7% of the total medical and diabetes admissions respectively. Within this period a total of 61 limb amputations were carried out and 26(42.6%) of these were DM related. The proportion of medical deaths due to "DMFS" deaths was greater than the proportion of medical admissions due to DMFS (p=0.007). The case fatality of subjects with DMFS was ~53%.
20 subjects with DMFS were hospitalized during the period of the study (2002–2003). The majority had type 2 DM. 65% of the total number of subjects with DMFS had some form of surgery in addition to medical management of their condition. The total costs incurred ranged from ^20,400:00 k to ^278,029:00 k. Drugs or medications accounted for the lion's share of the total costs incurred by the patients (46.9%).
Conclusion: The case fatality rate of diabetes foot ulceration and the subsequent costs arising from it are high. This report has shown the economic significance of this all important DM complication especially as occurring within a background of a resource poor country.

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Prevalence of polyneuropathy in impaired glucose tolerance and diabetes. The MONICA/KORA Augsburg Surveys and Myocardial Infarction Registry (KORA-A Study)

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Background and Aims: It is unclear whether there is a glycaemic threshold above which polyneuropathy develops. The aim of this study was to determine the prevalence of distal symmetric polyneuropathy (DSP) in two different populations in relation to the degree of hyperglycaemia.
Materials and Methods: KORA-A is based on participants from the population-based second and third MONICA/KORA Augsburg Surveys (S2+3) (representative samples aged 25–74 years) and the Augsburg Myocardial Infarction Registry (MIR). In 1997/1998, persons with diabetes who had been identified by these different sources were contacted (response rate about 65%) and assessed for the presence or absence of DSP by the Michigan Neuropathy Screening Instrument (MNSI) using a score cutpoint >2. An oral glucose tolerance test was performed in the controls. While S2+3 were population-based surveys, the MIR cohort comprised patients with a history of myocardial infarction.
Results: Among the participants from the S2+3 cohort, 194 persons had known diabetes (D: age [mean±SD]: 66.7 ± 9.4 yr, male sex: 57%), 46 had impaired glucose tolerance (IGT: 69.3 ± 7.8 yr, 50%), and 124 had normal glucose tolerance (NGT: 64.7 ± 9.1 yr, 57%). The corresponding numbers among the MIR participants were 213 (D: 68.9 ± 8.1 yr, 81%), 61 (IGT: 72.0 ± 6.8 yr, 72%), and 126 (NGT: 71.5 ± 6.5 yr, 75%). In the S2+3 cohort the prevalence of DSP was 27.6% in the diabetic subjects, 13.0% in those with IGT, and 8.9% in those with NGT (p<0.001 for D vs NGT, p=0.040 for D vs IGT, p=0.420 for IGT vs NGT). In the MIR cohort the prevalence of DSP was higher in each group, reaching 38.8% in the diabetic patients, 24.6% in the

IGT group, and 14.4% in individuals with NGT ($p<0.001$ for D vs NGT, $p=0.042$ for D vs IGT, $p=0.088$ for IGT vs NGT). Focusing on the IGT and NGT groups, pooling of the S2+3 and MIR data resulted in a significantly higher prevalence of DSP in individuals with IGT as compared to those with NGT (19.6% vs 11.7%; $p=0.047$, but $p=0.171$ after adjustment for sex and age).
Conclusion: At the population level the prevalence of polyneuropathy in individuals with IGT is slightly higher than in those with NGT. To establish whether this is a true difference, considerably larger samples would be required. Polyneuropathy is markedly more frequent in diabetic subjects than in those with IGT and more frequent among persons with a history of myocardial infarction than in the general population.

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Progress pattern of diabetic polyneuropathy and its influence on the prognosis
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Background and Aims: The initial manifestations, progression pattern and the impact on the prognosis of diabetic polyneuropathy (DPN) have not been fully understood. We aimed to obtain information to help in the early detection of impaired nerve function, the assessment of the severity of DPN, and the influences of DPN on mortality and morbidity by using the two independent investigations. The first investigation was carried out to search the early detection methods of impaired nerve function and to examine the progress mode of DPN in the cross-sectional study. The second investigation was done in order to observe the effect for the prognosis of DPN in the longitudinal study.
Materials and Methods: THE FIRST INVESTIGATION: Various somatic and autonomic nerve functions in forty diabetics and 20 age-matched healthy volunteers were evaluated using 7 objective examinations: nerve conduction study, quantitative vibratory perception threshold, heart rate variability, Valsalva test, head-up tilt test, Ice-water immersion test using photoplethysmograph and quantitative sudomotor axonal reflex test (QSART). The diabetics were divided into three groups according to the severity of microangiopathy. The nerve function data and prevalence of impairment were compared among the healthy control and three diabetic groups. THE SECOND INVESTIGATION: The influences of four somatic and autonomic nerve dysfunctions on the mortality and incidence of myocardial infarction, cerebral infarction and diabetic foot lesion were examined in 107 diabetics with preproliferative or proliferative diabetic retinopathy. They were followed-up after 4 nerve function tests, motor nerve conduction study, quantitative vibratory perception threshold, heart rate variability and Shellong test (orthostatic hypotenstion), for 1 to 15 years (average 5.1 years).
Results: THE FIRST INVESTIGATION: Results were as follows: 1) All nerve dysfunctions seemed to develop in parallel with the progression of microangiopathy, 2) reduced nerve conduction velocity and elevated vibratory perception thresholds in the feet might be early detectable signs of DPN, 3) vasomotor and sudomotor sympathetic functions and cardiovagal functions seemed to deteriorate with the appearance of microangiopathy, 4) compound muscle action potential lowering seemed to appear at the advanced microangiopathic condition, 5) hypohydrosis may most closely relate to diabetic foot ulcer. THE SECOND INVESTIGATION: Results were as follows: 1) Only the vasomotor sympathetic dysfunction (orthostatic hypotension) was related to the mortality and incidence of the foot lesion. 2) The relative risk of the death and foot lesion for control population of orthostatic hypotension group was odds ratio 5.08 and 10.33, respectively. 3) The sudden death cases were 57% (4/7) within the dead cases of orthostatic hypotension group.
Conclusion: DPN might generally progress with microangiopathy. Close relationships between sympathetic sudomotor and vasomotor dysfunctions and mortality, diabetic foot ulcer were suggested.

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The number of amputations as a quality marker of diabetic foot therapy – results after 5-year implementation of disease management project
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It's still a matter of international debate the fact that in years 1989–99 the number of amputations has not been reduced by 50%, and controversial opinion exists about the lack of precision and accuracy of data recorded as well as about the opportunity to consider the number of amputations as a quality marker in diabetic foot therapy. The aim of the present study is to evaluate whether the results obtained in 5-years implementation of ICDF (International consensus of Diabetic Foot) through a disease management project could contribute to better define the natural history of diabetic foot lesions in our district from the diagnosis to the final outcome. It has been considered as marker of qualità the prevalence and incidence of hospitalizations for diabetic foot lesions, and, among these, those exitated in amputations (DRG Tuscany), obtained by a retropective study performed in years 1996–1999 before DCI implementation, and those obtained by a prospective study (2000–03).The prospective study showed a progressive increase of ulcerative lesions examinated by the diabetic foot team in the hospital (337 in 1999, 969 in 2000, 1565 in 2001, 1744 in 2002, 2630 in 2003), associated with a 20% progressive reduction of hospitalizations/year and a significative decrease of total number of amputations, major amputation has occurred from 1999 to 2002(6.9×100000inhabitant to 1.8, $p<0.001$)from 2003 up to now the amputation rate remained stable. Moreover, our data indicate that, while in 1999 only 25% of patients from Pistoia area with diabetic foot problems were examinated in our hospital, progressively in following years this percentage has become higher (38% in 2000, 90% in 2001, 95% in 2002, 98% in 2003).It seems that the implementation of DCI can really contribute to reduce the number of amputations in diabetic subjects through a prevention program, follow-up and foot lesions treatment as well as it seems to influence disease costs through a reduction of hospitalizations. Moreover, a prospective study, made directly by specialists through an accurate data recording procedure concerning amputations can really contribute to a definitive validation of obtained data.

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Relation between alterations of peripheral nerve function and insulin resistance in non diabetic obese patients
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Background and Aims: Alterations of peripheral nerve function and cardiac autonomic tests are often found by the onset of type 2 diabetes. Some recent papers suggest that small peripheral nerve fibers may be altered in non diabetic obese patients. Cardiac autonomic dysfunction is also found in 30–50% of obese patients. Many obese women complain of symptoms consistent with nerve function impairment. The aim of this study was in non diabetic overweight or obese women to examine large fiber nerve function and to look for correlations with insulin resistance
Materials and Methods: Sixty-eight women aged 42 ± 3 years with BMI = 35.0 ± 1.4 kg/m^2 were included. Electrophysiological investigations included conduction velocities and amplitudes measurements for four peripheral nerves and reflex H measurement. The metabolic syndrome (MS) was defined according to NCEP/ATPIII criteria. Peripheral nerve dysfunction (PND) was defined according to reference criteria established in a large series of healthy subjects.
Results: MS was present in 14 patients, and PND in 32 patients. Blood glucose, plasma insulin, HOMA insulin resistance index, triglycerides and transaminases levels were significantly higher in the patients with PND ($p = 0.03$ to 0.005). PND was significantly more frequent in the patients with MS than in those without (71.4% vs 40.7%; $p = 0.04$) and in those with HOMA index > 3 than in those with HOMA index < 3 (68.4% vs 37.5%, $p = 0.03$).
Conclusion: These data show that the impairment of large nerve fiber function is highly prevalent among non diabetic obese women and suggest a link between such disorders and the metabolic syndrome and insulin resistance.

PS 94

Autonomic neuropathy, erectile dysfunction and cardiac function

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Left ventricular hypertrophy in type 1 diabetic patients: the influence of autonomic neuropathy

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Background and Aims: Left ventricular hypertrophy (LVH) is a prognostic factor for cardiovascular events in non-diabetic and diabetic patients. The mechanisms responsible for myocardial remodeling are not fully understood. The aim of the study was to determine the relative role of diabetic autonomic neuropathy in the development of LVH in type 1 diabetic patients.
Materials and Methods: 78 type 1 diabetic patients (30 M/48 F, age 28.9 ± 8.3 (SD) years, diabetes duration 9.7 ± 7.5 years) were investigated. The autonomic function was measured by standard cardiovascular tests (deep breathing and Valsalva maneouvre). 20 patients had normal autonomic function (DAN_0), 40 ones had early autonomic neuropathy (one positive or two borderline autonomic test results; DAN_1 group) and 18 patients had advanced autonomic neuropathy (two positive tests; DAN_2 group). Left ventricular geometry was defined by M-mode echocardiography. Left ventricular mass index (LVMI), relative left ventricular wall thickness (RWT), posterior wall thickness (PWT) and interventricular septum thickess (IVST) were estimated. LVH was diagnosed as LVMI >134 g/m^2 in men and >110 g/m^2 in women.
Results: The groups were similar by age, sex, HbA1c level, haemoglobin and proteinuria. The diabetes duration was higher in DAN_1 and DAN_2 groups as compared to DAN_0 group (10.6 ± 7.0, 11.7 ± 7.5 and 5.4 ± 5.0 years respectively, p=0.01). Deep breathing and Valsalva coefficient were decreased significantly in DAN_2-group as compared to DAN_0- and DAN_1-group (20.3 ± 8.0, 9.0 ± 5.2 and 5.4 ± 2.0, p=0.01; Valsalva test 1.26 ± 0.14, 1.16 ± 0.10 and 0.95 ± 0.29 respectively, p=0.01). There was significant increase in IVST and RWT parameters in DAN_2-group as compared to DAN_0- and DAN_1-group (IVS: 0.99 ± 0.17, 0.88 ± 0.16 and 0.82 ± 0.13 sm, p<0.05; RWT 0.4 ± 0.06, 0.37 ± 0.06 and 0.34 ± 0.04 respectively, p=0.05). The concentric type of LVH (RWT≥0.45) was revealed in 2 DAN_1- and 3 DAN_2- patients. The eccentric type was observed in 1 DAN_0-, 4 DAN_1- and 4 DAN_2-patients. The prevalence of LVH was greater in patients with DAN (5.9%, 15% and 43.8% in DAN_0, DAN_1, DAN_2 groups; χ^2=7.8, p=0.02). The heart rate, systolic and diastolic blood pressure were higher in patients with LVH (93 ± 12 vs. 82 ± 10 beats per minutes; 140.4 ± 21.5 vs. 123.1 ± 19.2 mm Hg; 90.4 ± 10.1 mm Hg vs. 76.9 ± 10.7 mm Hg respectively, all p=0.01). Left ventricular indexes negatively correlated with Valsalva test results (RWT r =-0.38, p=0.001; PWT r=-0.30, p=0.01; LVMI r=-0.27, p=0.025). Besides, LVMI correlated with the office systolic and diastolic blood pressure (r=0.36, p=0.005), proteinuria (r=0.26, p=0.034) and age (r=0.42, p=0.0001). In a multiple stepwise regression analysis age, male gender, tachycardia and Valsalva coefficient were associated with PWT and IVST increasing (R^2=0.55, p=0.018). Meanwhile, heart rate and Valsalva test parameter were predictors for RWT (R^2=0.47, p=0.027).
Conclusion: The obtained results demonstrate the relationship between autonomic function and left ventricular remodeling in type 1 diabetic patients. Autonomic neuropathy may increase cardiovascular risk in diabetes throughout development of LVH.

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Spanish Diabetic Autonomic Cardiac Neuropathy (DCAN) Multicentre Study Project. Proposal for diagnosis of the DCAN combining standard-cardiorespiratory parameters and HRV-spectrum frequency parameters

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Background and aim: We have previously shown date suggesting that RR-Variability spectrum frequency parameters using a short-term (5 min each, with the subject on supine and upright position) were significantly different in controls and diabetic patients classified as no-CAN by standard-cardiorespiratory parameters, and as it has been shown in some small samples a lack of association between the last and spectral parameters we decide to study both aspects in a larger sample of diabetic patients with and no-CAN.
Material and methods: The recording was obtained in a single session from the Valsalva manoeuvre (VMx2), deep breathing during a minute (DDBBx2), and two successive periods with the subject in supine and upright position of 5 minutes of duration each, from a sample of 512 diabetic patients, and from 97 controls. Date were reanalysed hardening the abnormality criterion for each standard-cardiorespiratory parameter (<2.5th percentile). This way no control subject had abnormal parameters.
Results: The overall DCAN prevalence was 28.4% (95% CI, 24–33%). A hierarchy for standard-cardiorespiratory parameters in its power to identify CAN was established (RMSSD>VP=Max-min=E: I). In supine position the HF-area (absolute units) was reduced in diabetes patients with only 1 cardio-respiratory parameter abnormal in relation to controls, and more in patients with ≥ 2 abnormal parameter (6.6–60.5 vs 27.1–266.5 vs 3.2–30.3 vs p <0.001). Higher was the LF-area reduction (in normalised units) when patient was in upright position (16.2–219.9 vs. 101.6–661.1 vs. 0.6–19.6 p <0.001). LF-area was also reduced in diabetes patients with no-CAN (34.4–389 vs. 101.6–661.1, p <0.001. The standard-cardiorespiratory parameters were associated among them. The RR-Variability spectrum frequency parameters were also associated among them (positive or negatively, depending if the tachographic-recording was obtained with the subject in supine or upright position). The association between both types of parameters was null or minimum.
Conclusions and diagnostic proposal for diabetic CAN: Although it requires a prospective study, we propose for diabetic CAN diagnosis a combination of two or more of the standard-cardiorespiratory abnormal parameters (mainly Max-min, VP and RMSSD) plus the HF and LF area with the subject in supine and upright position respectively.

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Cardiac autonomic neuropathy and erythropoietin level in type 1 diabetic patients

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Background and Aims: Anemia secondary to erythropoietin (EPO) deficiency is frequently observed in diabetic patients. The purpose of this study was to examine the influence of cardiovascular autonomic function, evaluated by 24 h blood pressure measurement and frequency domain analysis of heart rate variability (24-h Holter ECG), on EPO levels in diabetics.
Materials and Methods: The subjects include 34 type 1 diabetic patients (10 women and 24 men with a mean age of 45.1 ± 11.7 years, duration of diabetes 15.8 ± 9.5 years), who do not demonstrate significant renal disease. The subjects did not receive treatment other than insulin. Hemoglobin (Hb) and EPO concentrations were compared to those of non diabetic controls (N = 221). According to 24-hour ambulatory blood pressure measurement, subjects were divided in two groups; those with nighttime BP fall of more than 10% (dippers, N = 20) and those with less than 10% (non dippers, N = 14) when compared to daytime BP. Heart rate variability was evaluated using power spectral and temporal analysis of 24 h ECG Holter.
Results: The Hb and EPO levels of diabetic subjects were significantly lower than those of non diabetics (Hb: 14.1 ± 1 vs 14.6 ± 1 g/dl, p<0.04 and EPO: 11.7 ± 3.1 vs 14.3 ± 5.2 mU/ml, p<0.001). Hb levels were not significantly different among diabetic dippers when compared to controls (non-diabetics). However, EPO concentrations were significantly lower in dippers (11.3 ± 3.1 vs 14.3 ± 5.2 mU/ml, p<0.001). Hb levels were lower in non dippers when compared to dippers (13.7 ± 1.1 vs 14.5 ± 0.9 g/dl, p<0.04) but this difference was not associated with a significant increase in EPO levels. Patients' age, duration of diabetes, serum creatinine levels and anthropometric caracteristics did not differ significantly between dippers and non dippers. Frequency domain indexes were lower in non dippers than in dippers (log HF: 4,24 ± 1,34 vs 5.10 ± 0.99, p<0.05 and log LF: 6.03 ± 1.12 vs 6.99 ± 0.82, p<0.01). When compared to dippers, non dippers had a decrease in heart rate variability, a lower Hb level and a reduced EPO responsiveness to low Hb concentration. Hb levels were found to be negatively correlated with microalbuminuria (r=-0.397, p<0.02). No correlation between EPO levels and patients'age, duration of diabetes or glycated hemoglobin was demonstrated.
Conclusion: The results of our study confirm that EPO deficiency may exist in the absence of renal failure. We show that the absence of a nocturnal drop in BP in association with decreased heart rate variability causes a reduction in EPO levels. This study therefore confirms the link between autonomic nervous system dysfunction and erythropoiesis.

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Autonomic neuropathy is associated with hypertension in normoalbuminuric patients with type 2 diabetes mellitus

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Background and Aims: Cardiovascular autonomic neuropathy carries a five-fold risk for mortality in diabetic patients, but the precise explanation of increased mortality associated with autonomic neuropathy has not been identified. The aim of our study was to evaluate a possible relationship between autonomic neuropathy (AN) and 24-hour blood pressure profile in patients with type-2 diabetes mellitus.
Materials and Methods: The five standard cardiovascular reflex tests were used to assess autonomic function in 40 normoalbuminuric patients with type-2 diabetes who had no history of hypertension (mean age: 53,5 ± 7,2 years). Controls were 17 healthy control subjects (mean age: 41 ± 14,3 years). The severity of autonomic neuropathy was characterized by autonomic score. Ambulatory 24-hour blood pressure monitoring (ABPM) was performed by MEDITECH ABPM 04 device.
Results: The 24-hour mean systolic blood pressure values were significantly higher in diabetic patients with autonomic neuropathy compared to control subjects ($p<0,01$) and to diabetic patients without AN ($p<0,05$) even after adjustment for age, sex and smoking. Compared to patients without AN, those with AN showed also significantly higher blood pressure variability characterised by 24-hour systolic and diastolic blood pressure standard deviations (both $p<0.05$). Analyzing the relationship between ABPM parameters and the five cardiovascular reflex tests in the diabetic group as a whole, Valsalva ratio correlated negatively with the 24-hour systolic blood pressure standard deviation ($p<0.01$), and lower increase in diastolic blood pressure during the sustained handgrip test was associated with higher 24-hour diastolic blood pressure standard deviation ($p<0.05$). The increase of diastolic blood pressure during sustained handgrip correlated negatively with the 24-hour mean systolic and diastolic blood pressures (both $p<0,0001$). Blood pressure response to standing was inversely related to 24-hour mean systolic ($p<0,0001$) and diastolic ($p<0,01$) blood pressure values ($p<0,01$). There was a significant correlation between severity of autonomic neuropathy characterized by the autonomic score and 24-hour-long mean systolic ($p<0,0001$) and diastolic ($p<0,01$) blood pressure values. All these associations were not influenced by adjustment for age, sex, body mass index and smoking.
Conclusion: Autonomic neuropathy is independently associated with elevated 24-hour ABPM parameters and increased blood pressure variability even in normoalbuminuric patients with type-2 diabetes mellitus. ABPM is suggested to be performed for the early assessment of hypertension in diabetic patients with autonomic neuropathy and vice versa: autonomic function should be evaluated as part of the cardiovascular risk assessment in diabetic patients with hypertension. Higher 24-hour-long blood pressure values just as increased blood pressure variability may contribute to the poor prognosis of cardiovascular autonomic neuropathy in diabetic patients.

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Evolution of cardiac and gastric autonomic neuropathies in type 1 diabetes

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Background and Aims: The aim of this study was to assess the evolution of cardiac and gastric autonomic neuropathies (CAN and GAN) over a 3 years period in type 1 diabetic patients.
Materials and Methods: CAN and GAN were assessed over a three years period (Y0 and Y3) in 20 type 1 diabetic patients (mean age: 40.9 ± 9.5 years, diabetes duration: 12.8 ± 8 years) who did not receive other treatment than insulin and who were free of complications at Y0. Assessment of CAN was based on power spectrum analysis which was applied to 24 h ECG recordings to obtain total power (TP), low frequency power (LFP) and high frequency power (HFP). In the same time, gastric electrical activity was evaluated by cutaneous electrogastrography (EGG) spectral analysis and parameters selected were percentile of distribution of the 3 spectra of gastric slow-wave frequency: bradygastria for 0.5–2 cycles per minute (cpm), normogastria for 2-4 cpm and tachygastria for 4–10 cpm.
Results: Between Y0 and Y3 there was a significant decrease in HF spectral power (log HF: 5.13 ± 0.89 vs 4.36 ± 1.23, $p<0.03$) without any significant variation in LF. There was a significant increase in LF/HF ratio (5.60 ± 2.86 at Y0 vs 8.32 ± 4.03 at Y3, $p<0.02$). These results reflected a vagal dysfunction. Comparison between the 2 EGG shown a significant increase in percentage of tachygastria: 18.8 ± 9.4% at Y0 vs 25.4 ± 10.2% at Y3, $p<0.03$). This increase in tachygastria, which is due to vagal damage, was associated with a reduction in percentage of normogastria (75 ± 13.7% at Y0 vs 66.3 ± 13.2% at Y3, $p<0.05$). Between Y0 and Y3 variations in urinary albumine excretion rate was not significant (10 ± 13 mg/24 h at Y0 and 10 ± 10 mg/24 h at Y3).
Conclusion: Over a 3 years period we observe a reduction in heart rate variability which is associated in the same time with an increase in tachygastria. There is a parralelism in the evolution of CAN and GAN: the abnormalities observed are linked to a parasympathic damage and they occur before the others complications.

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Impact of the onset of erectile dysfunction on quality of life in patients with type 2 diabetes

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Background and Aims: In the context of the QuED project we longitudinally evaluated the impact of the development of erectile dysfunction (ED) on several aspects of quality of life (QoL) in patients with type 2 diabetes mellitus.
Materials and Methods: Patients were requested to fill in a questionnaire every 6 months for 3 years investigating the presence of ED and QoL (SF-36 Health Survey, depression symptoms [CES-D], and quality of sexual life). The analyses were based on multilevel models, adjusted for patient clinical and socio-demographic characteristics.
Results: The study involved 1456 patients, of whom 192 developed ED during the follow-up. For all the SF-36 dimensions, a deterioration in QoL preceded the development of ED. In particular, the deterioration in the physical functioning preceded on average by 6 months the decrease in the psychological well-being that in turn preceded by 6 months the onset of ED. The development of ED was clearly associated with a further decrease in all the scores, that reached the lowest level in concomitance with ED occurrence. Similarly, a worsening in depressive symptoms preceded the onset of ED, reached its highest level in concomitance with it, and tended to plateau thereafter. As expected, erectile problems were associated with a steep decline in the quality of sexual life. Multilevel models confirmed that the onset of ED was associated with a marked worsening in the physical functioning ($p=0.0008$), general health perception ($p=0.02$), and social functioning ($p=0.04$) SF-36 subscales, as well as in the summary SF-36 physical and mental components scores ($p=0.04$ and $p=0.07$, respectively). The development of ED was also associated with a highly significant increase in depressive symptoms ($p=0.001$) and a marked decrease in quality of sexual life ($p<0.0001$).
Conclusion: This longitudinal study documents for the first time that in patients with type 2 diabetes a deterioration in quality of life can represent an important alarm bell for the development of ED. The onset of erectile problems further worsens physical and psychological well-being. These findings highlight the importance of actively investigating ED problems in patients with type 2 diabetes, particularly in the presence of a recent functional decline or worsening of depressive symptoms.
Support: Pfizer Italiana S.p.A.

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Vardenafil 20-mg demonstrated superior efficacy to 10-mg in Japanese men with diabetes mellitus suffering from erectile dysfunction

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Background and aims: Vardenafil is a highly selective and potent phosphodiesterase type-5 inhibitor for the treatment of erectile dysfunction (ED). The efficacy of vardenafil has been demonstrated in a broad range of ED populations, but efficacy and tolerability in Japanese patients with diabetes mellitus (DM) had not yet been assessed. DM is frequently associated with difficult-to-treat ED. This is the first study to investigate the effectiveness of vardenafil in the subpopulation of Japanese men with DM and ED and to evaluate whether high-dose vardenafil (20 mg) can demonstrate superior efficacy to the usual dose (10 mg).

Materials and methods: The study was a 12-week, randomized, placebo-controlled, double-blind, multi-centre, parallel-group comparison study. Following a 4-week untreated observation period, 778 patients aged 26–64 years with ED and DM (patients with $HbA1_c$ >12% at screening were excluded) both for more than 3 years were randomly allocated to one of the 3 groups in a 1:3:3 ratio (placebo: 106, vardenafil 10 mg: 337, vardenafil 20 mg: 335). DM medications were continued throughout the study. The erectile function (EF) domain score of the International Index of Erectile Function was considered the primary efficacy parameter.
Results: Vardenafil 10 mg and 20 mg both significantly improved EF domain score from 13.6 and 13.9 at baseline to 20.8 and 21.9 at LOCF, respectively, versus placebo (13.68 at baseline and 16.28 at LOCF) ($p<0.0001$). Vardenafil 20 mg demonstrated superior efficacy to 10 mg ($p<0.05$), and this difference was more evident in patients with severe ED (baseline EF domain score <11). The safety profile was comparable between active treatment doses (drug-related adverse events: 6.6, 22.0 and 24.2% in placebo, vardenafil 10 mg, and 20 mg arms, respectively). The most common adverse events were hot flush, headache and nasal congestion, which were mild in intensity and transient, and are known to be common to PDE5 inhibitors. Incidence rates of adverse events leading to discontinuation of study medication were 1, 1, and 2% in the placebo, vardenafil 10 mg and 20 mg groups, respectively, while the incidence of serious adverse events was 0, 1, and 1%, respectively. None of the serious adverse events was considered related to study medication. There were no deaths during the study.
Conclusion: In Japanese men with DM and ED, vardenafil 10 mg and 20 mg were effective in improving erectile function with comparable safety profiles. Vardenafil 20 mg demonstrated superior efficacy compared with 10 mg, suggesting additional clinical benefit in using the higher dose in this difficult-to-treat population.
This study was fully supported by Bayer Yakuhin, Ltd.

PS 95
Complications, genetics

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Functional annotation of diabetic nephropathy genetic loci using kidney transcriptomic in diabetic rat models
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Background and Aims: Diabetic nephropathy (DN) is a multifactorial trait which results from the effect of sequence variants in several genes combined with unfavourable environmental factors. Linkage studies have identified several DN susceptibility loci that remain poorly characterised for functional candidate genes. The growing amount of genomic data in rodents and the development of functional genomic technologies are important resources for annotating human disease loci. We aimed to generate a comprehensive inventory of renal transcripts that are differentially expressed between diabetic rat models and map to chromosomal regions conserved with known DN loci in human 3q, 7q, 18q and 20p.
Materials and Methods: Kidney RNA samples were prepared from rat models of mild or severe hyperglycaemia exhibiting various renal histopathological defects (Goto-Kakisaki [GK] and Wistar-Kyoto [WKY] rats injected with Streptozotocin [STZ], respectively) and WKY controls. Probes were hybridised to both Affymetrix and custom arrays, which were used to quantify changes in the level of over 16,000 transcripts. Data interpretation was carried out using pathway analysis tools (Gene Ontology, KEGG and Ingenuity Pathway Analysis). EnsEmbl genome annotations were used for Rat-Human gene comparative mapping.
Results: Approximately 200 genes were significantly differentially expressed ($P<0.05$) in the kidney of GK rats when compared to WKY controls. Severe hyperglycaemia in WKY-STZ rats induced significant changes in the level of 682 and 833 transcripts when compared to WKY and GK rats, respectively. The transcript level of only 80 genes was consistently altered in both diabetic models when compared to WKY rats. The regulation of several gene pathways was altered in both WKY-STZ and GK rats, including those related to glycine, serine and threonine metabolism and tetrachloroethene degradation. Cellular growth and proliferation pathways were specifically affected in GK kidneys, whereas oxidoreductase activity was down regulated in WKY-STZ rats. Among the genes differentially expressed between diabetic models and the WKY control, 18 annotated genes and 15 ESTs map to regions of the rat genome conserved with known DN loci. Known genes include Glut2, Dgkg, CDC25B, Hrg, Rbp1, Slc14a2, Bdh, Clcn2, Pld1, Cldn16, Mep1b and Siat1.
Conclusion: Significant transcription regulation changes in diabetic models likely underlie the differential effects of mild and severe hyperglycaemia on metabolic pathways and/or histopathological renal changes. Combining rat transcriptomics and comparative genomics allowed an improvement in the functional annotation of human loci linked to DN by identifying diabetes reactive unannotated transcripts in the kidney of hyperglycaemic rats. These data provide strong experimental support for selecting novel target genes and functional pathways that can be tested in genetic association studies of DN.
This study is supported by Wellcome Trust and the European Commission

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Deficiency of mannose-binding lectin (MBL) attenuates the development of diabetic renal changes in a mouse model of type 1 diabetes
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Background and Aims: Diabetic nephropathy is the single-most important cause of end-stage renal failure in the Western World. An emerging amount of evidence supports the idea that the complement system and in particular mannose-binding lectin (MBL), plays a role in the development of diabetic nephropathy. To evaluate the specific role of MBL on the development of diabetic kidney disease, the impact of complete MBL-deficiency on diabetic renal changes was examined in an animal model of type 1 diabetes by the use of novel MBL double knockout (MBL-KO) mice.
Materials and Methods: 11-weeks-old MBL-KO female mice and wild type (WT)-female mice were divided into four groups: 1) non-diabetic WT-mice, 2) diabetic WT-mice, 3) non-diabetic MBL-KO mice and 4) diabetic

MBL-KO mice. Diabetes was induced by an intraperitoneal injection of streptozotocin (45 mg/kg body weight) in five consecutive days. Re-injections were preformed until the blood glucose level exceeded 16 mmol/l. After a diabetes duration of two months the animals were placed in metabolic cages for collection of 24-hours urine samples. Under anaesthesia the animals were sacrificed and the kidneys were weighed and further processed for later determination of glomerular volume by light microscopy and mRNA expression of connective tissue growth factor (CTGF) and collagen IV by quantitative RT-PCR.
Results: The diabetic WT-mice showed a pronounced increase in kidney weight and glomerular volume when compared to their non-diabetic controls; 164.5 ± 4.1 mg vs.118.0 ± 2.7 mg (mean±SEM), $p<0.001$ and 2.36 ± 0.07 $10^5 \mu m^3$ vs. 1.98 ± 0.09 $10^5 \mu m^3$; $p<0.005$, respectively. In contrast the diabetic MBL-KO mice showed no significant increase in kidney weight or glomerular volume when compared with their non-diabetic MBL-KO controls. MBL deficiency significantly attenuated the increase in urinary albumin excretion (UAE) and urinary albumin/creatinine ratio ($p<0.001$ and $p<0.02$, respectively). Renal CTGF and collagen IV mRNA expressions were significantly increased in diabetic WT-mice when compared with their non-diabetic WT-controls ($p<0.01$ and 0.001 respectively). In contrast renal CTGF mRNA expression in diabetic MBL-KO mice did not differ from the non-diabetic MBL-KO mice and the increase in collagen IV mRNA expression was attenuated in diabetic MBL-KO animals when compared to their controls ($p<0.002$). Blood glucose levels, body weight and food consumption did not differ between the diabetic groups.
Conclusion: Deficiency of MBL abolishes the diabetes-induced increase in kidney weight, glomerular volume and renal CTGF mRNA expression and attenuates the diabetes-associated increases in UAE and renal collagen IV expression. These findings are in support of the MBL-system playing a central role in the pathogenesis of diabetic kidney disease.
Support: The Danish Diabetes Association. The Danish Medical Research Council

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Beta-defensin and toll-like receptor expression in diabetic rats
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Background and aims: Many infections are associated with diabetes. Common sites of infection are the urinary tract and kidneys that could lead to nephropathy. Infections in diabetic people are frequently life threatening, as the ability of the body to fight infections is impaired. The innate immune system provides protection against a wide variety of pathogens. In humans, one mechanism of the innate immunity is the secretion of broad-spectrum antibacterial, cationic polypeptides (3–5 kDa) named defensins. Defensins also play a crucial role in modulating the adaptive immune responses. Toll-like receptors (TLRs) sense pathogen-associated molecular patterns and serve as another component of the innate immunity to identify bacterial invasion. Polymorphism in TLR2 and TLR4 has been associated with diabetes. Therefore, our objective was to determine β-defensin and TLR levels in the kidney in order to shed light on the general functionality of the innate immune system during diabetes.
Material and methods: Sprague-Dawley male rats were divided into 3 groups: 1) control; 2) diabetes-induced; 3) insulin-treated diabetes-induced. Chemical diabetes was induced by a single intramuscular injection of 65 mg/kg streptozotocin. Half of the rats with 250–300 mg/dL blood glucose levels received saline injections, whereas the other half received 3 units of Humulin R and 2 units of Insulatard HM in the morning and 1 unit of Humulin R and 5 units of Insulatard HM in the evening. After two weeks, rats were anesthetized with Nembutal (8 mg/Kg) and their kidneys removed. Kidney total RNA was subjected to Northern blot analysis using β-defensin 1 (rBD-1) and β-actin as probes. TLR levels were measured using quantitative real time PCR with GAPDH as a reference gene.
Results: Analysis of β-defensin levels by Northern blot in the diabetes-induced group (n=10) revealed only 64% ($p<0.002$) in the kidneys compared with the healthy group (n=6) **(Table 1)**. These low levels could be restored with insulin treatment (n=7) **(Table 1)**. Quantitative real-time PCR analysis demonstrated that TLR2 and TLR4 are highly expressed in normal kidneys. However, in accordance with the low levels of rBD-1, TLR2 and TLR4 levels were also low in diabetic rats and the levels could be restored with insulin treatment **(Table 1)**. Restoration of TLR4 level was not complete, but it was 2.6 fold higher than in diabetic rats **(Table 1)**.
Conclusions: Our results suggest that defensins and TLRs are poorly expressed in diabetic kidneys. This finding suggests that the innate immune system is indeed impaired and may explain the recurrent infections during diabetes. Further analysis of TLR levels in the kidney and other tissues in diabetic rats with and without insulin treatment will illuminate the molecular basis underlying the impairment of innate immunity. The data derived from this research may lead to future design of novel treatments that will induce defensins and TLRs and prevent recurrent infections in diabetic patients.

	Healthy Rats	Diabetic Rats	Insulin-treated Diabetic Rats
rBD-1 Levels	**100%**	**64%**	**95%**
TLR2 Levels	**100%**	**52%**	**140%**
TLR4 Levels	**100%**	**24.8%**	**64%**

Table 1: rBD-1 and TLR mRNA levels in healthy, diabetic, and insulin-treated diabetic male rats. The levels of rBD-1 mRNA in the kidney were analyzed by Northern blots, normalized using the levels of β-actin, and analyzed by the software ImageJ (version 1.32j). The levels of TLR mRNA in the kidney were analyzed by real time PCR and normalized using the levels of GAPDH. Results are presented as percentage compared to the healthy group.

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Increased risk for diabetic nephropathy in smokers carrying a polymorphism in the manganese superoxide dismutase gene
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Background and Aims: Oxidative stress has been suggested to contribute to the development of diabetic nephropathy (DN). Free radicals are formed in the mitochondria when the respiratory chain produces energy. To protect the cells there are important antioxidant enzymes, for example manganese superoxide dismutase (Mn-SOD). Mn-SOD is translocated into the mitochondrial matrix where it scavenges free radicals. In the targeting sequence of Mn-SOD there is a valine/alanine (V/A) polymorphism, which affects the translocation into the mitochondria. Hyperglycemia and smoking are known to increase the demand for antioxidants and, together with an impaired translocation of Mn-SOD, this can disturb the balance between antioxidants and radicals. The present case-control study aimed to determine if the V/A-polymorphism, alone or in combination with smoking, could contribute to development of DN in T1DM patients.
Materials and Methods: Patients with T1DM duration for more than 20 years, without albuminuria and without antihypertensive treatment (n=198) were considered as controls. Overt DN cases (n=48) were defined as patients having an albumin excretion rate (AER) >200 μg/min, in at least two overnight samples. AER between 20–200 μg/min, in at least two overnight samples, was defined as incipient DN (n=73). Patients answered a questionnaire on smoking habits and provided blood and urine samples.
Results: When adjusting for confounders (diabetes duration, HbA1c, age, smoking and sex), the Mn-SOD V/V genotype was significantly associated with a doubled risk for any DN. Present smokers homozygous for the V-allele, had five times higher risk to develop any DN compared to present smokers with A/A or A/V genotype. The risk for overt DN was 13 times higher among present smokers homozygous for the V-allele than among smokers with A/A or A/V genotype, results are presented in the table.
Conclusion: Our results indicate that homozygosity for the Mn-SOD V-allele can increase the risk for DN and this increase in risk is especially high among smokers.
Results: OR (95% CI) within different smoking groups

	Overt DN		Any DN		Controls	
	V/V	V/A+A/A	V/V	V/A+A/A	V/V	V/A+A/A
Smoked previously (n)	3	13	13	27	8	40
OR (95% CI)	1.2 (0.3–5.0)		2.4 (0.9–6.6)			
Smoking now (n)	6	4	8	13	2	18
OR (95% CI)	13.5 (2.0–93.2)		5.5 (1.0–30.5)			
Ever smoked (n)	9	17	21	40	10	58
OR (95% CI)	3.1 (1.1-8.8)		3.0 (1.3–7.1)			

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Genetic predisposition to complications of diabetes

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Background and Aims: Microvascular and macrovascular complications are responsible for the major long-term morbidity and mortality of both type 1 and type 2 diabetes. Although control of the abnormal metabolic state associated with both types of diabetes has a major effect on the incidence and severity of these complications, there is evidence, albeit inconsistent, that the propensity to develop these complications is, in part, genetically determined. We investigated the association between 4 single nucleotide polymorphisms in 3 candidate genes and complications of diabetes in a population with long-standing disease.
Materials and Methods: The blood samples and clinical data were collected from 1775 T1DM and T2DM Ashkenazi and Sephardic Jewish patients with 10 years or more known diabetes. Of these, 146 patients with missing critical clinical data were excluded leaving 1629 for analysis. DNA extracted from peripheral blood was genotyped for 2 MTHFR (C667T, A1298C) and one methionine synthase (A2756G) polymorphism by PCR-RFLP, and the ACE insertion/deletion polymorphism by duplex PCR. Genotype frequencies were compared between the patients with and without various diabetic complications.
Results: No statistically significant association was found between the ACE or the C677T MTHFR polymorphisms and any of the complications of diabetes. There was an association between the A1298C MTHFR polymorphism and nephropathy (p=0.018), evident predominantly in women (p=0.011). In addition, the MS A2756G polymorphism was related to diabetes-related eye complications, particularly in females with proliferative diabetic retinopathy (p=0.025). Interaction between MTHFR A1298G and MS A2756G is suggested, with patients carrying the risk genotypes at both loci having an increased prevalence of nephropathy (OR 3.4 [95% CI 1.1–11.1) or prevalence of any complication of diabetes (OR= 4.4 [1.1–29.0]), when compared to all other genotypes at these loci.
Conclusion: The previously reported association of the MTHFR C677T polymorphism with diabetic nephropathy was not confirmed; however folate levels were not determined. Our findings indicate that the A1298C polymorphism of MTHFR gene may be a risk factor for diabetic nephropathy in female Jewish patients, and that an interaction may exist between MTHFR and MS, both in the remethylation pathway.
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Methyltetrahydrofolate reductase C677T gene mutation and hyperhomocysteinemia as a novel risk factor for diabetic nephropathy

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Background and Aims: Hyperhomocysteinemia is a well defined risk factor for endothelial dysfunction and atherosclerosis. A point mutation (677C-T) of Methyltetrahydrofolate reductase (MTHFR) gene results in a significant increase at plasma homocystein levels. In this study we wanted to evaluate the effects of MTHFR gene mutation and consequent hyperhomocysteinemia on the development of diabetic microvascular complications in comparison with the other defined risk factors.
Materials and Methods: Diabetic patients without a history of macrovascular complication or overt nephropathy enrolled into the study. The presence of MTHFR 677 C-T point mutation was evaluated by Real-Time PCR technique by using a LightCycler. MTHFR heterozygous mutation was present in 24 patients over 52. Patients with diabetes were divided into two groups according the presence of MTHFR gene mutation. Both groups were well matched regarding age and diabetes duration. Metabolic parameters, plasma homocystein levels, microalbuminuria, plasma C-reactive protein (CRP), folic acid, and vitamin B12 levels were also studied. Presence of neuropathy and retinopathy were evaluated by specific tests.
Results: Duration of diabetes, body mass index, systolic and diastolic blood pressure, plasma CRP, A1c, and lipid levels were not different between the two groups. Plasma homocystein (12,89 ± 1,74 and 8,98 ± 1,91 μmol/L, p<0,0001) and microalbuminuria levels (73,40 ± 98,15 and 29,53 ± 5,08 mg/day; p=0,021) were significantly higher in the group with MTHFR gene mutation while creatinine clearance levels (91,11 ± 42,58 and 136,21 ± 51,50 ml/min; p=0,008) were significantly lower. Fourteen over 22 (65%) of the patients with microalbuminuria had MTHFR gene mutation, while this was only 26% (8 over 30) in normoalbuminuric patients (p=0,017). There was a significant correlation of plasma homocystein levels with creatinine clearance (r=-0,43;p=0,009) and microalbuminuria (r=0,33;p=0,05). We did not find any specific association of MTHFR gene mutation and hyperhomocysteinemia with retinopathy or neuropathy.
Conclusion: Plasma homocystein levels are significantly elevated in patients with MTHFR gene mutation. MTHFR gene mutation and consequent hyperhomocysteinemia were significantly correlated with microalbuminuria and decreased glomerular filtration rate. This was evident independent of other defined risk factors such as diabetes duration, hyperglycemia, and hypertension. As a result MTHFR gene mutation and consequent hyperhomocysteinemia might be accepted as an independent novel risk factor for diabetic nephropathy.

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Genetic variants of glucose transporter Glut-1 and renal involvement in type 2 diabetes

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Background and aims: In cell models, overexpression of Glut-1 gene induces mesangial expansion even in normoglycemia. Glut-1 XbaI genetic polymorphism was associated with nephropathy with conflicting results. No data regarding the association of additional variants and nephropathy are reported. We searched if genetic variants of Glut-1 in addition to the XbaI polymorphism were associated with renal involvement in type 2 diabetes patients.
Materials and methods: 1041 non related europid type 2 diabetes patients (409 women/632 men, aged 65 +/– 10 years, BMI 30.6 +/– 5.5 Kg/m^2) were recruited in an on-going prospective multicentre study. Diabetic nephropathy was staged according to anti-hypertensive treatment, serum creatinine levels and the highest three urinary albumin excretion in the ten previous years. Normoalbuminuria was defined as normal albumin excretion, no use of ACE-I or ARB, and either retinopathy or diabetes duration longer than 20 years. Microalbuminuria was defined as urinary albumin excretion in the range 20–200 mg/l or 30–300 mg/24 h with normal renal function. Proteinuria was defined as urinary albumin excretion >200 mg/l or 300 mg/24 h with normal renal function. Renal failure was defined as an estimated GFR below 30 ml/min using the Cockcroft formula. The stage of nephropathy could not be ascertained in 412 subjects.
Genetic determination of RS841853 was performed using XbaI restriction enzyme after PCR. Other genetic variants located in intronic regions were determined using the Taqman method.
Results: All genetic variants were in Hardy-Weinberg equilibrium. Renal disease was associated with Glut-1 genetic variants (table). Multivariate analysis using diabetes duration, HbA1c and systolic blood pressure did not modify the results.
Conclusions: Glut-1 genetic variants are associated with nephropathy severity in type 2 diabetic patients. Haplotype analyses and the search for functional signification of these variants must be performed.

	Normo-albuminuria	Micro-albuminuria	Protein-uria	Renal failure	p
SBP (mm Hg)	134+/-19	141+/-23	146+/-26	139+/-27	<0.0001
HbA1c (%)	7.9+/-1.3	8.0+/-1.4	8.0+/-1.3	7.4+/-1.3	0.0547
RS3738515					
n (%)	53(24)/104(47)/	55(31)/85(48)/	42(23)/101(56)/	8(16)/24(48)/	0.0407
CC/CG/GG	65(29)	37(21)	37(21)	18(36)	
RS3754219					
n (%)	57(25)/108(49)/	73(41)/77(43)/	48(27)/104(57)/	11(22)/87(54)/	0.0013
AA/AC/CC	57(26)	27(16)	29(16)	12(24)	
RS4658					
n (%)	145(65)/69(31)/	104(58)/63(36)/	119(66)/48(26)/	36(72)/12(24)/	0.2436
CC/CG/GG	8(4)	10(6)	14(8)	2(4)	
RS841853 (XbaI)					
n (%)	114(53)/84(39)/	63(36)/83(48)/	72(41)/90(51)/	22(44)/25(50)/	0.0039
TT/TG/GG	17(8)	28(16)	14(8)	3(6)	

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Genetic studies of diabetic nephropathy in three European populations (EURAGEDIC consortium): analyses of 14 candidate genes on chromosome 3q

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Background and Aims: Genetic factors influence the risk of developing diabetic nephropathy (DN). The objective of the European consortium EURAGEDIC is to identify DN susceptibility genes. Our strategy is based on large-scale case/control and intra-familial association studies of 150 candidate genes for DN. Linkage studies have mapped loci for DN and associated phenotypes on chromosome 3q. We have selected 14 genes located in this region for molecular genetic studies.
Materials and Methods: A total of 5152 DNAs have been assembled from 3 European populations in France, Denmark and Finland. These include: a) type 1 diabetic patients with (n=1588) and without (n=1356) DN for case/control studies, b) 571 trios (i.e. case or control proband and both parents) for intra-familial association studies (n=1472), c) non diabetic controls (n=736). The genes selected include (from 3-centromere to 3-qter): AGTR1, PTX3, IL12A, SLC2A2, TNFSF10, THPO, ECE2, EHHADH, KNG (kininogen), HRG, ACDC (adiponectin), SST, APOD, PPP1R2. After re-sequencing of the 14 genes in 188 chromosomes, 58 polymorphisms tagging the haplotypes with a frequency greater than 5% in at least one population were identified and further sought for association with DN. In each population, association was first tested by single-point analysis and followed by haplotype analysis performed on all SNPs within a gene. P values obtained from haplotype analyses carried out separately in the three populations were combined by use of the Fisher's method to produce an overall significance for association. Any suggestive association ($P<0.1$) was checked for confirmation through TDT analysis.
Results: Three polymorphisms showed suggestive association with DN in single-point analysis: the KNG 7965T [M178T] was at higher risk of DN (OR = 1.19 [1.05–1.34], $p < 10^{-2}$ in the pooled sample). The T allele also appeared over-transmitted in the TDT analysis (OR = 1.26 [0.96–1.64], p = 0.098). Two polymorphisms in the ACDC gene were shown to be associated with DN: an Ins/Del in the 3' flanking region and a G/A variant in the promoter region, the Del and the A alleles being respectively associated with increased risk (OR = 1.36 [1.13 - 1.64], $p <10^{-3}$ for the del allele and OR = 1.34[1.06–1.70], $p <10^{-2}$ for the A allele, in the pooled sample). TDT analyses confirmed the over-transmission of the A allele to patients with DN (OR = 1.81 [1.04 - 3.18], $p < 0.05$) but not of the del allele. No haplotype effect was observed.
Conclusion: We found suggestive association between DN and markers in 2 genes located on chromosome 3q27. Further investigations are needed to clarify the role of those genes in the pathogenesis of diabetic nephropathy.
This work was supported by a EEC grant (QLG2-CT-2001-01669) and Association Française des Diabétiques

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Peroxisome proliferator-activated receptor-gamma C161T polymorphism is not associated with type 2 diabetes and its complications

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Background and Aims: Peroxisome proliferator-activated receptor (PPAR)-gamma, a transcription factor in adipocyte differentiation, has important effects to insulin sensitivity, atherosclerosis, endothelial cell function and inflammation. Through these effects, PPAR-gamma2 might be involved with the type 2 diabetes and vascular disease, including diabetic complications. Recently, it has been reported that the C161T polymorphism in the exon 6 of PPAR-gamma is associated with type 2 diabetes interacting with uncoupling protein 2 (UCP2) gene, and it is associated with acute myocardial infarction. We have studied the association of this polymorphism with type 2 diabetes and its complications, such as retinopathy, ischemic stroke, nephropathy and neuropathy in a Korean non-diabetic and type 2 diabetic populations.
Materials and Methods: Three hundred and thirty eight type 2 diabetic patients (retinopathy: 64, ischemic stroke: 67, nephropathy: 39 and neuropathy: 76) and 297 healthy matched control subjects were evaluated. The PPAR-gamma C161T polymorphism was analyzed by PCR-RFLP.
Results: PPAR-gamma C161T genotype and allele frequency were not shown the significant differences between type 2 diabetic patients and healthy controls (T allele: 17.0 vs. 14.5, OR= 1.21, P=0.3188). In the analysis for diabetic complications, T allele in the ischemic stroke patients was higher than healthy controls, although it had no significance (T allele: 20.1 vs. 14.5, OR=1.49, P=0.1375). And in the multivariant regression analysis for clinical characteristics, including sex, age, BMI, hypertension and laboratory data, there were no significant associations.
Conclusion: These results suggest that the C161T polymorphism of the PPAR-gamma gene, by itself, is unlikely to influence type 2 diabetes and its complications.
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Metabolic syndrome and susceptibility to kidney disease

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Impact of insulin resistance/metabolic syndrome on glomerular filtration rate in patients with type 2 diabetes

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Background: The Insulin Resistance/Metabolic Syndrome (MS) is implicated in the pathogenesis of kidney disease in patients with type 1 diabetes. The role of the MS in the pathogenesis of kidney damage in patients with type 2 diabetes (T2D) is less clear. Indeed, no data are available on the impact of the MS on glomerular filtration rate (GFR) in these patients.
Aim: To investigate the role of the MS on GFR in a large cohort of patients with T2D.
Patients and methods: We enrolled 731 patients with T2D with mean age of age:61 ± 10 yrs and known duration of diabetes=11 ± 9 yrs. GFR was estimated (e-GFR) by the modified MDRD formula. Besides diabetes, the cardiovascular risk factors related to the MS were 1) arterial hypertension (SBP ≥ 130 and DBP ≥ 85 mmHg or current antihypertensive treatment); 2) dyslipidemia (patients on lipid lowering treatment or with total cholesterol ≥200 mg/dl, HDL cholesterol ≤40 mg/dl (M) and 50 mg/dl (F) and triglycerides ≥150 mg/dl); 3) abdominal obesity (waist circumferences >102 cm (M) and >88 cm (F)). Based on the number of cardiovascular risk factors present in each individual, a MS-related score (MS-rs)(from 0 to 3) was computed.
Results: MS-rs was 0 in 17 (2.3%), 1 in 86 (11.8%), 2 in 289 (39.5%) and 3 in 339 (46.4%) patients. In the whole cohort, e-GFR was 74.3 ± 19 ml/min/1.73 m^2 and was significantly correlated with gender (being lower in females, p<0.001) duration of diabetes (r=-0.3, p<0.001), urinary albumin excretion (r= -0.3 p<0.001), retinopathy (p=0.007), smoking status (r=0.1, p<0.001). e-GFR progressively decreased according to the increase in MS-rs (82 ± 19, 76 ± 18 and 71 ± 18 ml/min/1.73 m^2 in patients with score of 0–1, 2 and 3, respectively; p<0.001). This correlation was still significant after adjusting for gender (p=0.001), duration of diabetes (p<0.001), retinopathy (p<0.001) and the 3 variables considered together (p<0.001). The association was significantly stronger (p for interaction=0.002) in the subgroup with retinopathy, thus indicating interaction between retinopathy status (yes/no) and the MS-dc in modulating e-GFR and suggesting that, in T2D patients, micro-angiopathy strengthens the association between IR- related abnormalities and kidney function. A MS-rs of 3, significantly predicted the risk to have a e-GFR lower than 60 ml/min/1.73 m^2 (OR=1.8, 95% C.I.=1.1–2.8; p=0.014 adjusted for age and gender). The direct association between IR and kidney function was tested in an independent sample of 101 T2D who underwent euglycemic-hyperinsulinemic clamp and GFR measurements by ^{51}Cr-EDTA. A significant correlation was observed between the M and GFR values (r=0.31, p=0.002). This correlation was still significant after adjusting for gender (p=0.002), duration of diabetes (p=0.002), HbA1c (p=0.002), retinopathy (p=0.013) and the 4 variables considered together (p=0.011).
Conclusions: These data indicate that in patients with T2D, insulin resistance and the cluster of related abnormalities are strongly associated with reduced GFR. Our finding suggests that the MS is pathogenic for loss of renal function in T2D patients.

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Does obesity influence renal function in subjects with type 2 diabetes?

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Background and Aims: In non-diabetic subjects, there is good evidence that obesity leads to an increase in glomerular filtration rate (GFR) which exceeds smaller increases in renal plasma flow, thereby increasing filtration fraction. At the glomerular level, this is understood to be due to a dilatation of the afferent glomerular arterioles, which promotes an increase in single nephron GFR. These changes are identical to those known to occur in the early stages of diabetic nephropathy.
Materials and Methods: The aim of the present study was to assess whether GFR was associated with the body mass index (BMI) in a group of patients with type 2 diabetes attending a diabetes clinic in a university teaching hospital. GFR was measured using a reference method, the plasma disappearance of $^{99\,m}$Tc-DTPA and was also estimated using the following: creatinine clearance (CrCl), Cockcroft-Gault (CG)-formula and Modification of Diet in Renal Disease (MDRD)-6 formula. Adjustments for body surface area and BMI were made.
Results: As shown in the table, GFR estimated using the reference method, i. e. DTPA or calculated from the MDRD-6 formula was not associated with BMI. By contrast, estimates of GFR based on CrCl or CG-formula increased in parallel with increasing BMI. These increases were only partially corrected for by adjusting for body surface area.
Conclusion: In conclusion, results of this analysis of predominantly obese patients with type 2 diabetes do not support data obtained in non-diabetic subjects suggesting a link between obesity and renal function. In addition, the data suggest that caution should be used in interpreting estimates of GFR based on CrCl or the CG- formula.

Variable*	BMI <25 (n=81)	BMI 25–30 (n=217)	BMI 30–35 (n=165)	BMI >35 (n=125)	ANOVA p value
CrCl	85 ± 4	93 ± 3	97 ± 3	113 ± 5	< 0.0001
CG-formula	65 ± 3	79 ± 2	94 ± 3	122 ± 5	< 0.0001
MDRD-6-formula	73 ± 3	72 ± 2	72 ± 2	73 ± 3	0.98
DTPA-GFR	80 ± 3	82 ± 2	82 ± 3	86 ± 4	0.67

*Data are mean±SEM and expressed as ml/min/1.73 m^2

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The association of metabolic syndrome with nephropathy in type 2 diabetic subjects

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Background and Aims: Insulin resistance and other components of the Metabolic Syndrome (MS) have been implicated in the pathogenesis of diabetic nephropathy, but data about the relationship between metabolic syndrome and nephropathy in type 2 diabetes are spare. We aimed to evaluate the prevalence of MS and its relationship with microalbuminuria, overt nephropathy and renal function in a clinic-based cohort of subjects with type 2 diabetes.
Materials and Methods: 1314 type 2 diabetic patients participating in an ongoing single-centre study were included (58% males, 42% females; age 62+/–10 yrs and duration of diabetes 11.5+/–9.1 yrs). Urinary albumin was measured by an immunoturbidimetric method, serum creatinine enzymatically. Glomerular filtration rate was estimated by the simplified MDRD equation. The MS was defined according to NCEP ATPIII criteria. By definition, all subjects fulfilled the criteria for hyperglycemia. So, MS was defined adding any two of the following: abdominal obesity, increased triglycerides, low HDL, increased blood pressure. Based on A/C ratio patients were classified as having normoalbuminuria (nA: n. 1036), microalbuminuria (mA: n. 211, 16%) or overt nephropathy (ON: n. 67, 5%).
Results: The overall prevalence of the MS was 61% in males and 83% in females. The prevalence of MS increased with age but not with diabetes duration, and was tightly related to metabolic control: 59% in the lower quartiles of HbA1c (8.4%) (p<0.0001). The prevalence of central obesity was higher in women than in men (94 vs 52%). The same was for hypertension (71% vs 66%) and low HDL (40% vs 26%). High triglycerides had the same frequency in males and females (41 vs 43%).
Among males the prevalence of the mA and that of ON were higher in subjects with MS (23% and 8.5%) than in those with no MS (12% and 3.5%, p<0.0001). In females the same features were: MS (14% and 3.5%), no MS (3.5% and 1.2%, p=0.0075). Prevalence of raised albuminuria increased with the number of components of the metabolic syndrome (no components other than hyperglycemia, 1, 2, 3 and 4 components other than hyperglycemia) both in males (13%, 17%, 29%, 34% and 37%, respectively, p<0.0001) and in females (7%, 5%, 15%, 17%, and 24%, respectively, p<0.05).
Estimated GFR was lower in subjects with MS than in those with no MS (87+/–23 vs 92+/–20 ml/min*1.73 sqr m, p<0.001). This was true for both males (89+/–23 vs 93+/-21) and females (86+/-23 vs 89+/–18). When sub-

jects were stratified by the number of components of the metabolic syndrome, the lowest GFR value was in those with more severe (at least 4 traits) MS (83+/–23 ml/min/*1.73 sqr m, ANOVA Oneway p<0.0001) either males or females.
Conclusion: Metabolic syndrome, as defined by NCEP-ATPIII criteria, is very frequent in subjects with type 2 diabetes, more in females than in males. The prevalence of raised albuminuria levels (both microalbuminuria and overt nephropathy) is higher in subjects with metabolic syndrome and both in males and in females its frequency increase with the number of the components of the syndrome. By contrast, type 2 diabetic subjects without MS, although uncommon, are at low risk for renal (and likely cardiovascular) complications.

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Chronic kidney disease, metabolic syndrome and vascular complications in Chinese with type 2 diabetes: a cross-sectional study
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Background and Aims: Recently, metabolic syndrome (MES) has been suggested to be a risk factor for chronic kidney disease (CKD). Limited clinical data addressing the relationship between CKD, MES and vascular complications in type 2 diabetic patients. The aim of this study is to explore the associations between CKD, MES and vascular complications in type 2 diabetes.
Materials and Methods: Between 1995 to 2000, a consecutive cohort of 4984 type 2 diabetes referred to a university teaching hospital in Hong Kong underwent a comprehensive assessment of complications and cardiovascular risk factors based on the European DiabCare protocol. The frequency of CKD, as defined by a glomerular filtration rate (GFR) <60 ml/min/1.73 m^2, MES, as defined by the Asian modified criteria of National Cholesterol Education Program, macrovascular and microvascular complications and their associations were analyzed.
Results: In this study cohort, 16.1% patients had CKD, and 61.1% had MES. The percentage of patients with MES was significantly higher among those with reduced GFR (54.4% [95% CI 52.4–56.5%], 63.3% [61.1-65.5%], 75.0% [71.0–78.4%], 75.4% [67.9–82.9%], 81.4% [69.3–93.5%] for GFR≥90, 60–89, 30–59, 15–29, <15 ml/min/1.73 m^2 respectively (p<0.001). Decreasing GFR was significantly associated with progressive higher risk of macrovascular and microvascular complications of diabetes after adjusted for age, sex, smoking status, blood pressure, high-density lipoprotein, triglyceride, glycemic control (HbA_{1c} and fasting plasma glucose) in logistic regression analysis. Multivariate-adjusted odds ratios across different stages of GFR (≥90, 60–89, 30–59, 15–29, <15 ml/min/1.73 m^2) for macrovascular disease were 1.00, 1.17 [95% CI 0.89–1.52], 1.75 [1.27–2.41], 3.05 [1.87–4.97] and 4.11 [1.93–8.76] respectively (p<0.001); for retinopathy were 1.00, 1.34 [1.10–1.62], 2.53 [1.95–3.28], 4.66 [2.80–7.75] and 8.21 [3.37–20.01] respectively (p<0.001); for sensory neuropathy were 1.00, 1.41 [1.17–1.69], 2.13 [1.66–2.74], 4.78 [2.80–8.16]and 2.60 [1.18–5.77] respectively (p<0.001); for microalbumuria (with GFR <15 ml/min/1.73 m^2 excluded from the analysis) were 1.00, 1.24 [1.05–1.46], 4.54 [3.46–5.95] and 34.61 [10.89–110.9] respectively (p<0.001).
Conclusion: CKD and MES are common among Chinese type 2 diabetic patients. We demonstrated a positive relationship between MES and CKD, as well as a graded positive relationship between GFR and macrovascular and microvascular complications in Chinese type 2 diabetes. Our findings suggest CKD is a strong risk factor for both MES and vascular complications in diabetes. Further studies are required to explore the causal relationship between CKD, MES and vascular complications in type 2 diabetes.

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Metabolic syndrome and nephropathy in type 1 diabetes: the Italian cohort of the Eurodiab IDDM Complications Study
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Background and Aims: Prevalence of the MS and its relationship with microangiopathy are largely unknown in type 1 diabetes. We aimed to evaluate the prevalence of the MS in the Italian cohort of the EURODIAB IDDM Complications Study and to elucidate whether or not it is associated with microvascular complications.
Materials and Methods: This cohort consists of 978 type 1 diabetics (52% M, 48% F; mean age 32+/-10 yrs, diabetes duration (DD) 14+/–9 yrs; BMI 23.2+/–2.7 kg/m^2, HbA1c 8.1+/–1.8%). The MS was defined according to ATPIII criteria. By definition, all subjects fulfilled the criteria for hyperglycemia. So, MS was defined adding any two of the following: increased waist circumference, increased triglycerides, decreased HDL, increased blood pressure.
Results: Prevalence of the MS was 13.2% (12.0% in M; 14.6% in F). Hypertension was present in 27% of subjects (31% M, 23% F, p=0.005), high triglycerides in 10% (12% M, 8% F, ns), low HDL in 18% (16% M, 21% F, p=0.03) and abdominal obesity in 8% (3% M, 13% F, p<0.001). Three or more of the diagnostic criteria were observed only in 3.3% of subjects (3.0% M, 3.6% F), while 53% had no criteria other than hyperglycemia. Prevalence of MS increased with HbA1c quartiles (Q): 8.2% in Q1, through 10.7% and 14.7%, to 22.6% in Q4 (p<0.0001). High triglycerides (4.4% Q1, 6.1% Q2, 10.3% Q3 and 24.6% Q4, p<0.0001), low HDL (14.3% Q1, 17.6% Q2, 18.0% Q3 and 25.8% Q4, p=0.016), but not abdominal obesity (6.1% Q1, 6.9% Q2, 9.1% Q3 and 10.2% Q4, p=0.32) and high blood pressure (26.0% Q1, 26.7% Q2, 32.8% Q3 and 23.5% Q4, p=0.16) contributed to increase in frequency of MS with HbA1c. Prevalence of microalbuminuria (mA: 27% vs 18%) and overt nephropathy (ON: 21% vs 5%) were higher in subjects with MS when in those without (p<0.0001). In subjects with three or more factors of MS the rate of renal disease was not higher than in patients with only 2 factors other than hyperglycemia (mA: 32% vs 25%; ON: 19% vs 22%). By contrast, prevalences of mA (16%) and that of ON (1.2%) were very low in subjects with no MS factors other than hyperglycemia also adjusting for diabetes duration. Prevalences of background retinopathy (bR: 38% vs 31%) and proliferative retinopathy (pR: 22% vs 6%) were higher in subjects with MS when in those without (p<0.0001). In subjects with three or more factors of MS the rate of bR was not higher than in patients with only 2 factors other than hyperglycemia (32% vs 39%), while a difference was observed in pR (32% vs 19%). By contrast, prevalences of bR (26%) and pR (3.7%) were very low in subjects with no MS factors other than hyperglycemia also adjusting for diabetes duration. By stepwise regression analysis (factors of MS not included), MS (p<0.0001), duration (p=0.0005), cholesterol (p=0.005) and HbA1c (p=0.009) were independently correlated with nephropathy. Duration (p<0.0001) and MS (p=0.001) were independently correlated with retinopathy.
Conclusion: Metabolic syndrome, as defined by NCEP ATPIII criteria, is not a very frequent finding in italian type 1 diabetic subjects. Nevertheless, it is related to metabolic control and is likely to play a relevant and independent role in the development of microvascular complications.

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Non diabetic offspring of type 1 diabetic patients with nephropathy have a decreased insulin sensitivity
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Background and Aims: Previous studies had reported a decreased insulin sensitivity in type 1 diabetic patients with nephropathy and in their parents. Our aim was to determine if the non diabetic offspring of type 1 diabetic patients with nephropathy display a decreased insulin action.
Materials and Methods: Among families of type 1 diabetic patients aged >40 yr attending our department, 28 offspring accepted to participate to the study. Their glucose tolerance status was assessed by an OGTT. Percent fat mass was measured by dual energy X-ray absorptiometry. We measured total-body glucose utilisation rate using a euglycaemic hyperinsulinaemic clamp method (80 mUI/m^2/min). Offspring were identified in two groups according to whether their type 1 diabetic parent had an elevated albumin excretion rate (AER >30 mg/24 h) or a normoalbuminuria (AER <30 mg/24 h), without antihypertensive drug. Duration of diabetes was at least 20 years in all parents.
Results: Three offspring present an impaired glucose tolerance and were excluded for the analyses. Thus, we compared 7 offspring (4M/3F)) of type 1 diabetic patients with nephropathy with 18 offspring (6M/12F) of type 1 diabetic patients without nephropathy. The two groups were of similar age: 24,3 ± 4,7 vs 23,4 ± 5,4 yr (means±SD). Offspring of type 1 diabetic patients with nephropathy had a body-mass index higher than those of the type 1 diabetic patients without nephropathy: 25,1 ± 3 vs 21,6 ± 2,9 kg/m^2 respectively. However, the percentage of fat mass was similar in the two groups: 22,2 ± 7,3% for offspring of type 1 diabetic patients with nephropathy vs 21,4 ± 8,9% for offspring of type 1 diabetic patients without nephropathy.

Total- body glucose disposal rate was significantly lower in offspring of type 1 diabetic patients with nephropathy than those of diabetic patients without nephropathy: 9,77 ± 2,7 vs 13,97 ± 3,4 mg/kg of fat-free mass/min respectively. This difference remained significant after adjustment for sex and fat mass (p = 0.01). Systolic blood pressure was slightly higher in offspring of type 1 diabetic patients with nephropathy 125 ± 8 mmHg vs 115 ± 10 mmHg in offspring of type 1 diabetic patients without nephropathy (p= 0.02). Diastolic blood pressure was not significantly different: 71 ± 14 vs 66 ± 12 mmHg respectively.
Conclusion: In conclusion, non diabetic offspring of type 1 diabetic patients with nephropathy have a decreased insulin sensitivity. Our results suggest that diabetic nephropathy reflect an insulin resistance trait that is transmitted independently of diabetes.
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Blood pressure in non-hypertensive impaired fasting glucose (IFG) subjects
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Background and Aims: In 1997 American Diabetes Association (ADA) introduced impaired fasting glucose category (fasting plasma glucose 100–125 mg/dl), later adopted worldwide. In November 2003 ADA changed the lower threshold for IFG from 110 to 100 mg/dl, the change being still a matter of controversy. Hypertension is common in diabetes patients, however, the association between those recently defined glucose tolerance disorders and elevated blood pressure is unclear. The aim of this cross-sectional study was to assess the relation between borderline glucose intolerance and blood pressure in non-hypertensive individuals.
Materials and Methods: Eight hundred and fifty randomly selected non-hypertensive non-diabetes middle-aged individuals aged >45 (mean age 61.0 ± 10.9 years) were enrolled into the study. Full medical examination with fasting plasma glucose (FPG) and lipids assays were performed in all the participants. Blood pressure was measured after 10 min rest, the result noted was a mean of three measurements. The subjects were divided in six subgroups according to mean fasting plasma glucose values: Group 1 – FPG <80 mg/dl (n=204); Group 2 – 81–89 mg/dl (n=181); Group 3 – 90–99 mg/dl (n=144); Group 4 – 100–109 mg/dl (n=119; new subset of IFG category); Group 5 – 110–125 (n=147; previous IFG range); Group 6 – >125 mg/dl (n=55; diabetes).
Results: Mean (±SD) systolic blood pressure in each group was 131 ± 22, 131 ± 21, 133 ± 21, 137 ± 20, 143 ± 20, 143 ± 21, respectively, and diastolic blood pressure values were 80 ± 12, 80 ± 11, 83 ± 12, 82 ± 10, 84 ± 11, 83 ± 13 mm Hg, respectively. Systolic blood pressure was significantly higher in Groups 4–6 than in Groups 1-3 ($p<0.05$), while there was no significant difference found in diastolic blood pressure values. Mean age of subjects in each group was: 62, 59, 59, 62, 65, 64 years ($p>0.05$), body mass index 26.8, 26.9, 28.2, 29.4, 30.8, and 30.9 kg/m^2 ($p<0.05$), fasting plasma triglycerides 1.84, 1.74, 1.83, 2.08, 2.13, 3.28 mmol/l ($p<0.05$), total cholesterol 5.43, 5.44, 5.39, 5.46, 5.6, and 5.45 mmol/l ($p>0.05$), HDL cholesterol 1.21, 1.26, 2.02, 1.18, 1.17, and 1.09 mmol/l ($p>0.05$), LDL cholesterol 3.4, 3.65, 3.32, 3.34, 3.49, 3.28 mmol/l ($p>0.05$), respectively. The rise in systolic blood pressure was associated also with increasing body mass ($r=0.2135$; $p<0.05$) and plasma fasting triglycerides ($r=0.2133$; $p<0.05$).
Conclusion: Systolic blood pressure in IFG subjects, also in the newly added category (FPG 100–109 mg/dl), is significantly greater than in the subjects with normal fasting plasma glucose. As new larger category of IFG encompasses subjects with rising systolic blood pressure, body weight as well as plasma triglycerides, it may become an appropriate target for metabolic syndrome and type 2 diabetes prevention programmes.
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Risk factors of subjects with pre-hypertension in metabolic syndrome
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Background and Aims: The aims of the study are to investigate clinical features and the risk factors of pre-hypertensive subjects to develop metabolic syndrome.
Materials and Methods: The study enrolled a total of 5,948 subjects from the Department of Health Management of Chang Gung Medical Center. Of the 5,948 subjects, 1,532 subjects were diagnosed with metabolic syndrome. Whole body three-dimensional (3-D) laser scanner scans were used for the anthropometric measurements. The health index (HI) was determined by 3-D data. According to JNC-7 criteria, pre-hypertension was defined as an SBP of 120–139 mmHg or a DBP of 80–89. The subjects are categorized into four groups. Group A (1,401 cases) is younger than 65 years old, without metabolic syndrome and with pre-hypertension. Group B (337 cases) is older than 64 years old, does not have metabolic syndrome with pre-hypertension. Moreover, group C (297 cases) is younger than 65 years old and has metabolic syndrome, pre-hypertension. Finally, group D (100 cases) is older than 64 years old and has metabolic syndrome and pre-hypertension.
Results: Of the four groups in this study, the ratio of DM, overweight, and WHR progressively elevated from A to D. WBC count differs statistically significantly between metabolic and non-metabolic groups. Anthropometric measurement illustrated higher BMI and percentage of overweight subjects in the young groups (A and C); otherwise WHR and HI show higher in ageing (B and D). Gender and ageing differences were noted in subjects of non-metabolic syndrome and metabolic syndrome with pre-hypertension. Female percentage illustrated a marked increased in group D comparing with other groups. The incidence of DM diagnosed among the four groups is similar to the presentation of gender. Univariate and multiple regression analysis demonstrates TG/HDL ratio, HI, fasting sugar, gender, UA, LDL, WBC, and WHR are independents factors for the pre-hypertension in metabolic syndrome.
Conclusion: DM percentage, overweight, and gender are important factors associated with risk of metabolic syndrome with pre-hypertension in ageing.

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Manidipine (but not amlodipine), increases insulin sensitivity and rises plasma adiponectin concentrations in hypertensive non-diabetic patients with metabolic syndrome and impaired fasting glucose
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Background and Aims: Hypertensive patients with metabolic syndrome are at a very high risk of developing diabetes mellitus. Many trials have shown that the choice of antihypertensive drugs may affect the incidence of diabetes, but few (if any) studies have been conducted specifically in patients with metabolic syndrome. The aim of this work is to study the possible differences in the effects of two calcium channel blockers on insulin sensitivity in these patients.
Materials and Methods: 64 patients with essential hypertension (naïve, or after 1 month washout), impaired fasting glucose, and at least one of the three other NCEP ATP-III criteria for metabolic syndrome (HDL <40/50 mg/dl, triglycerides >150 mg/dl and waist perimeter >88/102 cm, M/F) were recruited. Previously unknown diabetes mellitus was excluded by OGTT. Weight, height, waist perimeter were measured by standard methods, blood pressure and heart rate with a validated semiautomatic device (OMROM M4I) in standard conditions, and plasma glucose, HbA_{1C}, creatinine, Na^+, K^+, AST, ALT, lipid profile, hs-CRP and adiponectin, and albumin excretion rate (in an overnight collection) were measured by routine laboratory methods. The insulin resistance index was calculated by the HOMA model. The patients were randomized to manidipine 20 mg (M) vs. amlodipine 10 mg (A) daily; after 8–12 weeks all measurements were repeated, compliance was assessed by pill counting, and tolerance by questionnaire.
Results: One patient was withdrawn in the A group because of severe ankle oedema; all the rest completed the protocol. The compliance was good in both groups, with >90% of the patients having taken >80% of the pills. One patient in the M group and 3 patients in the A reported minor side effects (M: ankle oedema; A: 2 ankle oedema, 1 headache). The systolic blood pressure was reduced by 16.2 + 4.3 (A) vs. 15.9 + 5.0 (M) mmHg (p NS between

treatments, p < 0.001 vs. baseline); the diastolic blood pressure was reduced by 9.2 + 4.8 (A) vs. 8.8 + 4.5 (M) mmHg (p NS between treatments, p <0.001 vs. baseline); the albumin excretion rate was significantly (p = 0.003) reduced by 37.3% (CI 95%: 23.4 to 59.4) by M, but not significantly by A (7.9%; CI: –3,8 to 19.7); p = 0.009 between treatments. Plasma hs-CRP was reduced significantly (p<0.01) by both treatments: 13.7% (CI: 5.3 to 22.8) with M and 14.9% (CI: 6.7 to 24.6) with A, p NS between treatments. Plasma adiponectin was significantly increased by M (32.9%, CI 19.3 to 45.6%, p = 0.008) but not by A (4.8%, CI –7.4 to 16.0), p = 0.011 between treatments. The HOMA insulin resistance index was very significantly reduced by M (-21.3%, CI –16.6 to –28.7, p = 0.007) but with A the reduction was close to signification (–8.3%, CI -17.4 to 0.3, p = 0.062); p = 0.010 between treatments. The reduction in the HOMA insulin resistance index correlated strongly with the increase in plasma adiponectin (Pearson's R = 0.58, p <0.001). The other studied variables remained unchanged.

Conclusion: Both treatments were equally effective in blood pressure reduction, with lower incidence of side effects with Manidipine. Only Manidipine was effective in the reduction of the albumin excretion rate, while both reduced hs-CRP in a similar fashion. The improvement of insulin sensitivity in significantly better with Manidipine, and is associated with an increase in plasma adiponectine.

PS 97

Nephropathy, inflammation and oxidative stress

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C-reactive protein is associated with high-normal urinary albumin excretion in normotensive and normoalbuminuric type 1 diabetic patients

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Background and Aims: An association of low-grade inflammatory markers with diabetic nephropathy has been proven in type 1 diabetic patients.
As it is well known that the degree of albuminuria is closely related to the progression of nephropathy, we aimed to determine whether C- reactive protein and homocysteine value significantly differs between type 1 diabetic patients with high normal and low normal urinary albumin excretion.

Materials and Methods: Two hundred and ten normoalbuminuric (urinary albumin excretion <30 mg/24 h measured at three occasions) and normotensive (casual BP <130/80 mmHg at three occasions) type 1 diabetics were included. They were divided upon the albumin excretion median (16,2 mg/24 h) into low-normoalbuminuric (n=116) and high-normoalbuminuric group (n=94). None showed signs of renal or cardiovascular disease and received antihypertensive or other drugs that could attenuate low-grade inflammation.
Urinary albumin concentration was determined by an immunoturbidimetric assay. C reactive protein was measured by radioimmunoassay. Homocysteine was measured by chemiluminescence's technique.

Results: The low-normoalbuminuric (UAE= 8,02 ± 4,56) and high-normoalbuminuric group (UAE= 23,41 ± 6,16, p=0.0001) did not differ regarding age, gender, BMI, HbA1c, systolic and diastolic blood pressure, HDL cholesterol, triglycerides, creatinine clearance, smoking status. High-normoalbuminuric in comparison to low-normoalbuminuric group showed significantly higher duration of diabetes (18,2 ± 9,18; 14 ± 8,98; p=0.003) and C- reactive protein value (2,17 ± 4,94;1,17 ± 2 mg/l; p=0.04). Homocysteine was also higher in the high-normoalbuminuric compared to low-normoalbuminuric group, but the level did not reach the statistical significance (9,53 ± 7,64; 9 ± 2,89 µmol/l; p=0.56). Urinary albumin excretion was positively correlated with diabetes duration, BMI, triglycerides, C-reactive protein, homocysteine, diastolic blood pressure and inversely with HDL. C-reactive protein was positively correlated with diabetes duration, BMI, triglycerides, urinary albumin excretion, homocysteine and inversely to HDL.
In a multiple regression analysis with diabetes duration, BMI, HDL, triglycerides, urinary albumin excretion, homocysteine and HDL cholesterol in a model, C-reactive protein was independently associated with urinary albumin excretion (β=0,103), homocysteine (β=0,259) and diabetes duration (β=0,63; p=0.001).

Conclusion: C reactive protein, a low-grade inflammatory marker, is associated with high-normal urinary albumin excretion in type 1 diabetic patients. The independent relation of diabetes duration with C-reactive protein suggests that chronic exposure to glucose may stimulate its production already at the high-normoalbuminuric stage. Whether the detection of increased C-reactive protein value in normoalbuminuric type 1 diabetics has predictive value for development of microalbuminuria needs to be assessed.

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Low-grade inflammation is correlated to diabetic nephropathy in type 2 diabetes mellitus

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Background and Aims: Increased urinary protein excretion is the earliest clinical finding of diabetic nephropathy. Low-grade inflammation is, according to various studies, present to type II diabetic patients. The aim of our study was to investigate the hypothesis that low-grade inflammation – assessed by plasma levels of CRP and IL-6 – is associated with diabetic nephropathy.

Materials and Methods: We included to our study 72 (M/F 44/28, age 48 ± 13.8 years) type II diabetic patients with microalbuminuria (Group A)

and 55 (M/F 32/23) type II normoalbuminuric diabetic patients (Group B) matched by age, sex, systolic and diastolic blood pressure. We excluded patients with acute illness on course, recent myocardial infarction and other factors interfering with IL-6 levels. Each patient underwent to a complete laboratory evaluation and estimation of urinary albumin excretion, plasma levels of CRP and IL-6. Multiple regression analysis was applied.
Results: Patients with microalbuminuria had statistically higher levels of CRP (1.1 ± 0.3 mg/dl - group A vs. 0.4 ± 0.06 mg/dl - group B, $p<0.05$) and interleukin-6 (3.91 ± 0.7 pg/ml – group A vs. 1.8 ± 0.6 pg/ml – group B, $p<0.01$). Multiple regression analysis showed that there was a significant positive correlation between microalbuminuria and plasma levels of IL-6 ($p<0.001$), CRP ($p<0.05$).
Conclusion: According to our data, low-grade inflammation, reflected by IL-6 and CRP levels, is present in the early stage of diabetic nephropathy. Plasma levels of IL-6 are correlated with microalbuminuria. Perhaps this reflects the participation of inflammation process in the pathogenesis of diabetic nephropathy.

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Serum interleukin-18 levels is associated with albuminuria and atherosclerosis in patients with type 2 diabetes

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Background and Aims: Recent studies have suggested that inflammatory mechanisms are involved in the pathogenesis of diabetic nephropathy as well as atherosclerosis. Interleukin-18 (IL-18) is a pro-inflammatory cytokine secreted from mononuclear cells. The aim of this study is to assess whether serum IL-18 level is a predictor of diabetic nephropathy in patients with type 2 diabetes.
Materials and Methods:A total of 82 Japanese patients with type 2 diabetes (48 women and 34 men) and 20 control subjects (13 women and 7 men) were enrolled. The patients with renal dysfunction (creatinine clearance <60 ml/min) were excluded from this study. We examined the clinical parameters, serum and urinary IL-18 levels, serum IL-6 levels, serum levels of soluble form adhesion molecules, sVCAM-1, sICAM-1, sP-selectin, sE-selectin, and sL-selectin. Carotid intimal-media thickness (IMT) and brachial-ankle pulse wave velocity (ba-PWV) were also measured. Changes of urinary albumin excretion rate (AER) in 6 months were assessed in 76 diabetic patients. Logarithmic transformation of serum or urinary IL-18 levels, serum IL-6 levels, high-sensitive C-reactive protein (hs-CRP), AER, serum soluble adhesion molecules was used to render the distribution normal for the parametric tests. A P value <0.05 was accepted as indicating statistical significance.
Results:Serum IL-18 levels were higher in patients with type 2 diabetes than in age and sex-matched control subjects (179 ± 62 vs. 146 ± 46 pg/ml, $P = 0.024$). By univariate linear regression analysis, we found a significant positive correlation of serum IL-18 levels with AER ($r = 0.53$, $P < 0.0001$), urinary β-2 microglobulin ($r = 0.24$, $P = 0.036$), HbA1c ($r = 0.24$, $P = 0.029$) and hs-CRP ($r = 0.24$, $P = 0.031$). We also found a significant correlation between urinary IL-18 levels and AER ($r = 0.31$, $P = 0.005$). Multivariate linear regression analysis showed that AER (partial coefficient = 0.41, $P <0.0001$) and hs-CRP (partial coefficient = 0.21, $P = 0.033$) were independently associated with serum IL-18 levels. AER was also independently associated with urinary IL-18 levels (partial coefficient = 0.30, $P = 0.005$). Serum IL-18 levels also positively correlated with carotid IMT and ba-PWV ($r = 0.23$, $P = 0.042$ and $r = 0.23$, $P = 0.040$, respectively). Univariate analysis showed that serum IL-18 levels correlated with the serum levels of sICAM-1, sP-selectin, and sE-selectin ($r = 0.29$, $P = 0.009$, $r = 0.31$, $P = 0.006$, and $r = 0.23$, $P = 0.014$, respectively). Moreover, the AER was significantly increased within 6 months in patients with high IL-18 levels (>178 pg/ml) as compared with the patients with low IL-18 levels (<177 pg/ml) ($P = 0.018$).
Conclusion: Serum IL-18 levels are closely associated with AER in patients with type 2 diabetes. Serum IL-18 levels might be a predictor for early stage of diabetic nephropathy as well as atherosclerosis in type 2 diabetes.
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Monocyte Chemoattractant Protein-1 (MCP-1) has direct prosclerotic effects in human mesangial cells

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Background and Aims: Inflammatory mechanisms are believed to contribute to the pathogenesis of diabetic glomerulosclerosis. Monocyte Chemoattractant Protein-1 (MCP-1), a potent chemokine, is overexpressed in the glomeruli from diabetic animals and in human mesangial cells exposed to high glucose and mechanical stretch. The MCP-1 receptor, CCR2, is predominantly expressed in monocytes, but has been recently demonstrated in mesangial cells. We studied in human mesangial cells, whether MCP-1 has a direct prosclerotic effect mediated by the CCR2 receptor. Specifically, we investigated if MCP-1 binding to CCR2 alters fibronectin and TGF-β1 production and the intracellular mechanisms involved.
Materials and Methods: Human mesangial cells were serum-deprived for 24 hours, then exposed to increasing rh-MCP-1 concentrations (10, 100, 200 ng/ml) for 12, 24, and 48 hours. Fibronectin and TGF-β1 protein levels were measured by ELISA. The intracellular mechanisms were investigated using a panel of specific inhibitors or blocking antibodies [CCR2 inhibitor (RS102895, 6 μM); anti-TGF-β1 blocking antibody (2 μg/mL); NF-kB inhibitor (SN50, 18 μM); P38-MAPK inhibitor (SB202190, 1 μM), and PKC inhibitor (peptide 19–36, 4 μM)]. NFkB p65 active levels were measured using the TransAM kit.
Results: rh-MCP-1 induced a significant increase in fibronectin protein levels at 24 hours (12 hours: 0.78 ± 0.1; 24 hours: 2.5 ± 0.4; 48 hours: 0.8 ± 0.1, fold increase over control; $p<0.01$ MCP-1 at 24 hours). This effect was also seen at the lowest MCP-1 concentration of 10 ng/ml (10 ng/ml: 1.95 ± 0.26; 100 ng/ml: 2.49 ± 0.41; 200 ng/ml: 2.08 ± 0.02, fold increase over control, $p<0.01$ at all concentrations) and it was specific as it was completely abolished by the CCR2 inhibitor RS102895 (MCP-1:2.2 ± 0.01, MCP-1+RS102895: 1 ± 0.1, fold increase over control, $p<0.01$ MCP-1 vs others). MCP-1-induced fibronectin was mediated by TGF-β1 as MCP-1 induced a significant increase in TGF-β1 protein levels at 12 hours (12 hours: 2.1 ± 0.3; 24 hours: 1.5 ± 0.1; 48 hours: 1.3 ± 0.1, fold increase over control, $p<0.05$ MCP-1 vs control at 12 and 24 hours) and TGF-β1 blockade completely prevented the fibronectin response to MCP-1 (MCP-1: 1.78 ± 0.2; MCP-1+anti-TGF-β1: 1.13 ± 0.2, fold increase over control, $p<0.05$ MCP-1 vs others). MCP-1-induced TGF-β1 was not altered by either PKC or P38-MAPK inhibition, whereas it was significantly reduced by SN50 (MCP-1: 2.2 ± 0.3; MCP-1+SN50:1.3 ± 0.13, MCP-1+PKC19-36: 2.01 ± 0.18, MCP-1+SB202190: 1.97 ± 0.19, fold increase over control, $p<0.05$ MCP-1, MCP-1+PKC19-36, MCP-1+SB202190 vs control and MCP-1+SN50). MCP-1 addition to HMC significantly induced NFkB p65 active levels at 30 minutes (MCP-1: 1.51 ± 0.1, fold increase over control, $p<0.005$).
Conclusion: MCP-1 binding to the CCR2 receptor induces fibronectin production via a NF-kB-TGF-β1-dependent mechanism, confirming the hypothesis of a direct prosclerotic effect of the MCP-1/CCR2 system in human mesangial cells. This represents a possible mechanism by which MCP-1 may contribute to glomerulosclerosis in diabetic and other progressive glomerulopathies.

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Mechanisms of hyperhomocysteinemia in T2DM with nephropathy

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Background and Aims: Hyperhomocysteinemia is a common finding in type 2 diabetes mellitus (T2DM) with nephropathy and is a risk factor for vascular disease. The pathophysiological mechanism(s) of this alteration are not known.
Materials and Methods: To this aim, seven male non obese T2DM patients with albuminuria (about 1 gram per day) and mild to moderate renal insufficiency (plasma creatinine: 1.3 mg/dl), and seven age- and BMI-matched non diabetic controls with normal renal function, were infused with L-[methyl-$^2H_3$, 1-^{13}C]-methionine for 6 hours. Rates of homocysteine clearance through remethylation and transsulfuration, as well as of methionine appearance (both methyl- and carbon-), transmethylation and disposal into proteins, were determined both before and following an euglycemic, hyperinsulinemic (about 1000 pmol/L) clamp. Both a plasma and an intracellular model were applied.

Results: The T2DM patients had a 2-fold greater homocysteine concentrations than controls ($p<0.01$). In the T2DM, homocysteine clearance through both transsulfuration and remethylation was decreased by >50% up to 6-fold than in controls ($p<0.01$ or less). Transmethylation was also significantly decreased in the T2DM patients. The ratio between transmethylation and transsulfuration was however not different between groups. The insulin-induced increments of transsulfuration, transmethylation and homocysteine clearance through transsulfuration were markedly diminished (by >3 fold) in the T2DM subjects with respect to controls ($p<0.01$ or less). In contrast, endogenous methionine appearance in the T2DM patients was not greater than in the controls.
Conclusion: The hyperhomocysteinemia of T2DM patients with nephropathy is likely due to a decreased homocysteine clearance (particularly through transsulfuration and, to a lesser extent, through remethylation), and not to an increased production from methionine. These defects are further amplified by hyperinsulinemia.

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Prevention of microvascular complications of diabetes by high dose S-benzoylthiamine monophosphate (benfotiamine): mechanism of thiamine delivery into cells
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Background and Aims: The thiamine prodrug Benfotiamine is under current clinical investigation for the prevention of microvascular complications of diabetes. It is thought to deliver thiamine into cells due to its increased lipophilicity - although as a phosphate ester, like thiamine it has a low partition coefficient. Benfotiamine is a derivative of thiamine monophosphate (TMP). Recent studies in experimentral diabetes in vivo with Benfotiamine showed increased plasma and tissue concentrations of TMP – indicating that Benfotiamine may be a vehicle for TMP and may itself enter cells by the transporter used by TMP, the reduced folate carrier-1 (RFC-1). The aim of this study was to examine of Benfotiamine enters cells via the RFC-1 transporter.
Materials and Methods: Human red blood cells (RBCs), 50% cell suspension, in Krebs-Ringer phosphate buffer, pH 7.4 and 37 °C, were incubated with 50 μM Benfotiamine for 2 h and the effect of the RCF-1 ligand methotrexate (10 μM) on thiamine delivery examined. Thiamine, TMP and thiamine pyrophosphate (TPP) were assayed by HPLC with fluorimentric detection after pre-column derivatisation to thiachromes.
Results: Incubation of RBCs with Benfotiamine gave a 4–5 fold increases in RBC thiamine, TMP and TPP. Methotrexate prevented the increases in TMP and TPP but a 4-fold increase in thiamine remained. The concentration of thiamine in RBCs was (mean±SEM, n = 4; pmol/ml packed RBCs): control 172 ± 38, + Benfotiamine 907 ± 45 ($P<0.001$), + methotrexate 535 ± 71 ($P<0.01$) and Benfotiamine + methotrexate 740 ± 20 ($P<0.001$). The concentration of TMP in RBCs was (mean±SEM, n = 4; pmol/ml packed RBCs): control 22.5 ± 8.6, + Benfotiamine 89.4 ± 9.6 ($P<0.01$), + methotrexate 6.3 ± 3.2 and Benfotiamine + methotrexate 33.1 ± 1.8. The concentration of TPP in RBCs was (mean±SEM, n = 4; pmol/ml packed RBCs): control 24.1 ± 4.6, + Benfotiamine 113.1 ± 23.9 ($P<0.05$), + methotrexate 44.4 ± 20.6 and Benfotiamine + methotrexate 42.5 ± 1.1. Incubation of RBCs with Benfotiamine and exogenous thiamine (50 μM) increased RBC thiamine only a further 5-fold, possibly by benzoyl exchange from Benfotiamine to extracellular thiamine and delivery of thiamine into cells by S-benzoylthiamine.
Conclusion: Benfotiamine utilises the methotrexate inhibitable RFC-1 transporter predominantly to increase cellular concentration of TPP via delivery of TMP. This is consistent with recent experimental observations in vivo showing increased plasma and tissue concentrations of TMP with high dose therapy with Benfotiamine but not with high dose therapy with thiamine, although both increased cellular concentrations of TPP and prevented incipient nephropathy with similar potency.

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Ramipril and telmisartan reduce inflammation and lipid peroxidation in diabetics
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Background: Angiotensin-converting enzyme inhibitors (ACE-I) and angiotensin II type 1 receptor blockers (ARB), have a well established beneficial role in the treatment of diabetes mellitus (DM).
We tested whether ramipril (an ACE-I) and telmisartan (an ARB) have comparable antioxidant and anti-inflammatory effects in patients with DM.
Methods: Fourty patients, 57.3 ± 8.7 years old (17 men, 23 women), with well controlled type 2 DM without hypertension, ischemic heart disease or microalbuminuria were enrolled in this double blind cross-over study. They received ramipril 2.5 mg/day or telmisartan 40 mg/day for 3 months. Subsequently, after a wash up period of 2 weeks they were crossed over to the alternative treatment for another 3 month period. Finally, all patients received both medications for 3 more months again after a 2 weeks wash out. High-sensitivity C-reactive protein (hsCRP) and oxidized LDL-cholesterol plasma levels were measured at baseline and at the end of each treatment period (i.e. at 3, 6 and 9 months). Statistical analysis was done with Friedman's analysis of variance.
Results: both hsCRP and oxidized LDL were substantially reduced by all treatments.

	hsCRP (mg/dl)	Oxidized LDL-cholesterol (mU/l)
Baseline	0.21 ± 0.15	11.7 ± 4.2
Ramipril	0.16 ± 0.15*	8.5 ± 3.3‡
Telmisartan	0.13 ± 0.10*	9.6 ± 3.3‡
Combination therapy	0.12 ± 0.10*	7.8 ± 2.5‡

*$p<0.001$ compared with baseline hsCRP
‡$p<0.001$ compared with baseline oxidized LDL- cholesterol
None of the above regimens affected blood pressure or blood glucose levels

Conclusion: Telmisartan is equally effective as ramipril, with their combination being even more potent, in counteracting inflammation and lipid peroxidation in patients with DM.

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Hypertension exaggerates renal oxidative stress but not inflammation in the early stage of experimental diabetes mellitus
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Background and Aims: Combination of diabetes and hypertension increases the incidence and severity of kidney disease in an additive manner. Individually diabetes and hypertension is able to promote renal inflammation and oxidative stress. These last two conditions have been implicated in the mechanism of renal lesion in both diabetes and hypertension. However, the exact sequence of events by which inflammation and oxidative stress interact to exacerbate diabetic renal disease with concomitant hypertension is largely unknown. The aims of the present study were to identify if renal inflammatory and oxidative stress events are additive from the beginning in diabetic animals with coexisting hypertension, and if the regulation of oxidative and inflammatory process differs between hypertensive diabetic animals and their normotensive controls.
Materials and Methods: Diabetes (D) was induced in spontaneously hypertensive rats (SHR) and their genetically normotensive control Wistar Kyoto (WKY) rats by streptozotocin at 12 weeks of age. After 10 days, rats were killed and kidneys were collected. Renal macrophage (ED1) infiltration and oxidative DNA damage (8-hydroxy deoxyguanosine, 8-OHdG) was evaluated by immunohistochemistry. Intra-nuclear fraction of p65 subunit of nuclear factor-κB (NF-κB) and nitrotyrosine levels were determined by Western blot. Reduced glutathione (GSH) levels were quantified by chemical method.
Results: Plasma glucose levels were similar in WKY-D and SHR-D groups. The systolic blood pressure was higher in both groups of SHR rats than WKY groups. Glomerular and tubulointerstitial macrophage infiltration (ED1 positive cells per glomerular cross section (gcs) and per high power field (hpf) respectively) was significantly higher in WKY-D (1.81 ± 0.51 vs 1.04 ± 0.26 cells/gcs, $p<0.05$; 22.60 ± 4.45 vs 8.88 ± 2.15 cells/hpf, $p<0.001$) and SHR-D (2.65 ± 0.22 vs 1.75 ± 0.32 cells/gcs, $p<0.01$; 24.04 ± 8.12 vs 11.33 ± 1.04 cells/hpf, $p<0.01$) groups than the respective control (C) groups. Surprisingly, activation of NF-κB showed a strong tendency to be reduced in both diabetic groups (WKY-D and SHR-D) than their controls, indicating an NF-κB-independent mechanism of renal inflammation in the early stage of diabetes. Non-enzymatic antioxidant GSH level was similar in WKY-D (4.42 ± 0.2 μM/g tissue) and WKY-C (4.26 ± 0.36 μM/g), but it was significantly lower ($p<0.05$) in SHR-D (3.71 ± 0.18 μM/g) than SHR-C (4.09 ± 0.3 μM/g). Oxidative stress-induced DNA damage, as measured by 8-OHdG, was found similar in WKY-C and WKY-D, but it was significantly elevated in SHR-D as compared with SHR-C. Nitrotyrosine expression, a marker of oxidative and nitrosative stress, was also similar in WKY-C and

WKY-D, but it showed a strong tendency to be elevated in SHR-D in comparison to SHR-C.
Conclusion: These findings indicate that short duration of diabetes mellitus can induce renal inflammation in both WKY and SHR strains, and that coexisting hypertension is unable to induce additional influence on renal inflammation. However, combination of diabetes and hypertension clearly exacerbates renal oxidative stress. Therefore, renoprotective effect of early correction of hypertension in diabetes may come, at least partly, through modulation of redox balance. Presently we are investigating to confirm our preliminary data concerning NF-κB and nitrotyrosine, and the underlying basis of their contribution in diabetic nephropathy.
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Erythromycin ameliorates renal injury through anti-inflammatory effects in experimental diabetic rats
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Background and Aims: Recent studies have shown that the inflammatory process is involved in the pathogenesis of diabetic nephropathy. Fourteen-membered ring macrolides, including erythromycin, have anti-inflammatory effects as well as antibacterial effects. The aim of this study is to investigate whether erythromycin is protective against diabetic nephropathy through its anti-inflammatory effects in a model of streptozotocin (STZ)-induced diabetic rats.
Materials and Methods: Sprague-Dawley (SD) rats were divided into following three groups: *1*) non-diabetic group (ND; n=6), *2*) STZ-induced diabetic group (DM; n=6), and *3*) treatment group in which diabetic rats were treated with erythromycin (DM+EM; n=6). Rats in the DM and DM+EM groups at the age of 5 weeks received intravenous injections with STZ at 65 mg/kg BW. Erythromycin (5 mg/kg BW) was orally administrated daily to rats in the DM+EM group, starting from 7 days before STZ injection. Eight weeks after induction of diabetes, all rats were sacrificed and the kidneys were harvested. To evaluate the effects of erythromycin treatment, we assessed urinary albumin excretion (UAE), histological changes, the expression of intercellular adhesion molecule-1 (ICAM-1), infiltration of macrophage and nuclear factor-kappa B (NF-κB) activity in the kidney.
Results: There were no significant differences in blood pressure and creatinine clearance among the three groups. UAE in the DM group progressively increased at 4 weeks ($p<0.05$) and 8 weeks ($p<0.005$) compared with the ND group. However, the treatment with erythromycin significantly reduced UAE by 48.2% at 8weeks ($p<0.05$).
Immunofluorescence staining for ICAM-1 in glomeruli and immunoperoxidase staining for ED-1 (macrophages) were performed. The expression of ICAM-1 was significantly higher in the DM group than in the ND group ($p<0.0001$) and it was significantly reduced by erythromycin treatment ($p<0.0001$). The number of ED-1-positive cells was significantly higher in the DM group than in the ND group ($p<0.0001$). Infiltration of macrophages was significantly suppressed by treatment with erythromycin ($p<0.0001$) (ND *vs.* DM *vs.* DM+EM: 0.2 ± 0.03 *vs.* 1.6 ± 0.08 *vs.* 0.9 ± 0.07).
The mRNA expression of TGF-β1 in renal cortex was evaluated by quantitative real-time RT-PCR. TGF-β1 mRNA expression in the DM group was 1.6-fold higher than in non-diabetic rats ($p<0.01$). Erythromycin treatment reduced the increase of TGF-β1 mRNA expression ($p<0.05$). Immunofluorescence staining for type IV collagen revealed that type IV collagen was significantly increased in the DM group than in the ND group ($p<0.0001$) and that in the DM+EM group, it was markedly decreased than in the DM group ($p<0.0001$).The activation of NF-κB was analyzed using electrophoretic mobility shift assay (EMSA). NF-κB activation in the kidney was higher in the DM group than in the ND group and it was significantly reduced by treatment with erythromycin ($p<0.005$).
Conclusion: Erythromycin prevented renal injuries without changes of blood glucose levels and blood pressure in experimental diabetic rats. Our results suggest that the renoprotective effects of erythromycin are based on its anti-inflammatory effects via suppression of NF-κB activation. Modulation of microinflammation with erythromycin may provide a new approach for diabetic nephropathy.

PS 98
Nephropathy, clinical

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Kidney size and pulsatility index predict loss of renal function in type 2 diabetic patients
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Background: The decline in renal function is eterogenous in type 2 diabetic (D2) patients (pts) and the reasons for this variability is poorly understood. Aims: To determine the prognostic value of renal ultrasonographic imaging and Doppler analysis of renal interlobar arteries on the decline of renal function in D2.
Methods: Prospective observational study of 42 (30 males) D2 pts aged 56.4 ± 7.7 years (yrs) (mean±S.D.) followed for median 7.2yrs (range 2–14yrs). At baseline clinical features (CF), glomerular filtration rate (GFR) by the plasma clearance of 51Cr-EDTA, albumin excretion rate (AER) on three 24 hour urine collections were evaluated. By ultrasound color Doppler we measured Kidney size (KV) and Pulsatility index (PI) of renal interlobar arteries. At every follow up (Fu) visit (4–15; range of number of visits per pt) CF, GFR and AER were determined.
Results: At baseline BMI was 28.3 ± 4.2 kg/m^2, known duration of diabetes (DD2) 11.3 ± 6.9 yrs. 14 pts had never smoked and 8 were actual smokers. Systemic blood pressure was 147 ± 15 / 85 ± 9 mmHg, AER 57 (2 to 3125) μg/min [median(range)], HbA1c 8.4 ± 1.6%, serum cholesterol (CT) 226 ± 43 mg/dl, triglycerides (TG) 194 ± 108 mg/dl. 31% of the pts had background diabetic retinopathy, 17% proliferative retinopathy. Renal function was on average in the normal range: GFR 100 ± 27 mL/min/1.73 m2. 8 pts had normal AER (<20 μg/min), 20 pts had incipient (≥20<200 μg/min) and 14 pts had overt nephropathy (≥200 μg/min). Mean values of KV (305 ± 86 ml) and PI (1.38 ± 0.30) were clearly above the normal range (normal values: KV 219 ± 71 ml, PI 0.9 ± 0.17; p<0.01 for both). There was a significant correlation between PI and AER (0.402, 0.008) [R; p] and between KV and baseline GFR (0.374; 0.018). During Fu mean rate of decline of GFR (ΔGFR) was -3.99 ± 5.62 mL/min/1.73 m^2/year, mean systemic blood pressure $145 \pm 10/84 \pm 5$ mmHg, HbA1c 8.5 ± 1.1%, CT 214 ± 42 mg/dl and TG 187 ± 99 mg/dl. ΔGFR correlated significantly with PI (-0.42; 0.005) [R; p], baseline AER (-0.5; 0.001), mean Fu systolic blood pressure (-0.35; 0.021), baseline CT (-0.38; 0.012), mean Fu CT (-0.449; 0.003), BMI (-0.37; 0.017) and DD2 (-0.31; 0.041). We devided pts in progressors (Pr) [12 pts] and non progressors (nPr) when they were above and below the tertile value of distribution of ΔGFR (-5.5 ml/min/1.73 m^2/year). Pr had significantly larger KV than nPr (357 ± 43 vs 285 ± 89 ml; p=0.016) and higher PI (1.5 ± 0.34 vs 1.3 ± 0.26; p=0.026). Pr had higher baseline GFR (114 ± 30 vs 95 ± 25 ml/min/1.73 m2/year; p=0.034) and higher baseline AER (57 (2-3125) vs 43 (2-671) μg/min); p=0.011). Pr had a longer DD2 (14.8 ± 8.1 vs 9.8 ± 6.0yrs; p=0.037), higher basline CT (251 ± 51 vs 216 ± 35 mg/dl; p=0.015) and Fu CT (252 ± 52 vs 199 ± 27 mg/dl; p=0.000). Absence of retinopathy was signficantly prevalent in nPr than in Pr (86 vs 14; %, p=0.025).
Conclusions: This data demonstrate that: 1. PI is significantly correlated with AER, while KV is correlated with GFR. 2. Both PI and KV are predictive of the subsequent decline in GFR. Although further studies are necessary to understand the nature of these relationships, we suggest that renal ultrasound imaging could be helpful in identify pts at high risk of progression.

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Reduced aortic distensibility in diabetic, compared to non-diabetic, patients with end-stage renal disease
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Background and Aims: Aortic distensibility (AD) is an early marker of atherosclerosis and predicts survival in both the general population and in patients with end-stage renal disease (ESRD). AD is reduced in diabetes. However, no data exists on the effect of diabetes on AD in patients with ESRD. In this study we examined differences in AD between patients with ESRD according to the presence or not of diabetes.

Materials and Methods: A total of 55 patients with creatinine clearance <20 ml/min (24 with diabetic nephropathy; mean age 60.3 ± 15.1 years, and 31 patients with renal failure due to causes other than diabetes, mean age 57.6 ± 16.4 years) were examined. In addition, 20 healthy controls, matched for age and sex, with the renal patients were also recruited. A percentage of 40% of the diabetic patients and 48% of the non-diabetic patients were on chronic hemodialysis. AD and Left Ventricular Mass index (LVMi) were assessed by high-resolution ultrasonography.
Results: The patients with ESRD had lower values of AD as compared to controls (1.60 ± 0.16 vs 2.31 ± 0.34 $cm^2\ dyn^{-1}\ 10^{-6}$, respectively, P< 0.001). LVMi was almost double in the patients with ESRD than the controls (201.7 ± 46.9 vs 118.8 ± 21 g/m^2, respectively, P< 0.001). The values of AD were significantly lower in the patients with diabetic nephropathy than in the patients with nephropathy from other causes (1.53 ± 0.12 vs. 1.66 ± 0.16 $cm^2\ dyn^{-1}\ 10^{-6}$, respectively, P= 0.002). No significant differences were found in LVMi between ESRD patients with and without diabetes (201.2 ± 46.4 vs 202 ± 48.2 g/m^2 respectively, P=0.96).
Conclusion: AD is reduced in patients with ESRD. In addition, the presence of diabetes exerts an additional detrimental effect on the elastic properties of the aorta. This finding may explain in part the increased cardiovascular mortality in the diabetic patients with ESRD.

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Renal hyperfiltration in type 2 diabetes: effect of age related decline in glomerular filtration rate
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Background and Aims: To characterize the effect of the age-related decline of glomerular filtration rate (GFR) on the prevalence of hyperfiltration (HF) in type 2 diabetes and to identify clinical characteristics associated with HF.
Materials and Methods: GFR was measured in 662 type 2 diabetic patients by the plasma disappearance of ^{99m}Tc-DTPA. Prevalence of HF was calculated using both an age-unadjusted GFR threshold of $>130\ ml{\cdot}min^{-1}{\cdot}1.73^{-2}$ and an age-adjusted threshold incorporating an age-related decline of $1\ ml{\cdot}min^{-1}{\cdot}year^{-1}$ after the age of 40 years. The HF patients were compared with Type 2 diabetic subjects with a GFR between 90–130 $ml{\cdot}min^{-1}{\cdot}1.73\ m^{-2}$ matched for age, gender and disease duration to allow for identification of modifiable factors associated with HF.
Results: The prevalence of HF was 7.4% when age-unadjusted and 16.6% when age-adjusted definitions were used. The age-unadjusted vs age-adjusted prevalence rates for HF were 50% vs 50%, 12.9% vs 23.4% and 0.3% vs 9.0% for patients aged 65 years, respectively. Both the age-unadjusted and age-adjusted HF groups had lower diastolic blood pressures (DBP) and lower serum creatinine levels in comparison with the control groups. Although the age-unadjusted HF group had larger kidneys compared to the control group, this difference was no longer significant when the age-adjusted definition was used. There were no differences in HbA1c, mean arterial pressure, antihypertensive use, insulin therapy, lipid levels, frequency of macro- or microvascular complications, body mass index and urinary sodium, urea and albumin excretion between the groups.
Conclusion: HF was still more common among younger patients with type 2 diabetes even after adjusting for the expected age-related decline in GFR. HF is associated with lower DBP independent of age.
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Calculation of glomerular filtration rate (GFR) from plasma cystatin C in type 1 and type 2 diabetes
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Background and Aims: Several studies and data produced by ourself suggest that cystatin C is superior to creatinine as a marker of GFR in diabetic subjects. Cystatin C more promptly detects subtle decrements of renal function. GFR is calculated from creatinine using the Cockcroft-Gault (C-G) formula or the MDRD equation. The MDRD equation has many advantages, but does not overcome the low sensibility of creatinine. We compared the GFR (iohexol plasma clearance) with cystatin C with two aims: 1. to confirm cystatin C as a more sensitive indicator of early impairment of renal function; 2. to calculate an equation to convert cystatin C to GFR.
Materials and Methods: A group of 226 diabetics (122 type 1, 104 type 2; 141 M, 85 F) was recruited. Among type 1 (age 37+/–9 yrs, BMI 23.9+/–2.8 kg/m^2, HbA1c 8.5+/–1.5%, DD 21+/–9 yrs), 31% had normal AER, 33% microalbuminuria, and 36% overt nephropathy. The respective features for type 2 (age 60+/–8 yrs, BMI 27.7+/–4.2 kg/m^2, HbA1c 7.9+/–1.5%, DD 12+/–11 yrs) were 12%, 60% and 28%. GFR was estimated by plasma clearance of iohexol (Omnipaque 300 - Nycomed, Oslo). Serum creatinine was measured enzymatically and serum cystatin C by the Dade/Behring PENIA test on a BN II analyzer.
Results: In type 1 diabetics sCr was 1.28+/–0.66 mg/dl (range 0.60–5.27), cys-C 1.15+/–0.59 mg/l (0.31–3.18), C-G 80+/–33 (21–141) ml/min, MDRD 69+/–25 (13–128) ml/min, and I-GFR 88+/–42 ml/min/1.73 m^2 (16–222). In type 2 diabetics, the respective values were 1.35+/–0.91 (0.65–6.38) mg/dl, 1.04+/–0.54 (0.40–3.32) mg/l, 94+/–49 (12–174) ml/min, 72+/–30 (7–131) ml/min, and 85+/–38 (5–166) ml/min/1.73 m^2. Relationships between I-GFR with the reciprocal of cys-C was stronger than those with the reciprocal of sCr, C-G and MDRD in type 1 (r=0.90, r=0.75, r=0.69 and r=0.78, p<0.001, respectively) but not significantly in type 2 (r=0.84, r=0.80, r=0.81 and r=0.84, ns). Diabetics were then divided in subjects with „high“ or „low“; renal function (arbitrary thresholds 80 ml/min). In subjects with „low“ function all parameters were related to I-GFR. On the contrary, in „high“ function patients, correlation between I-GFR and sCr, C-G and MDRD were lost in type 1 diabetics and weak in type 2, while the correlation with cys-C remained significant in both type 1 and type 2. ROC plots suggest that the AUC for cys-C is greater than those of sCr, C-G, and MDRD both in type 1 and in type 2. Comparisons between areas become significant when ROC analysis (I-GFR cut-off 100 ml/min) was restricted to subjects with „high“ GFR: AUC for cys-C was significantly greater than those for sCr, C-G and MDRD in type 1 and marginally so in type 2. By power regression analysis, a correlation curve was calculated between I-GFR and cys-C and the following equation obtained: GFR = 72.493 × (Cys C)–1.158. Very similar equations were obtained when correlation curves were calculated separately for type 1 (GFR = 67.501 × (Cys C)–1.100) and type 2 diabetics (GFR = 76.194 × (Cys C)–1.188).
Conclusion: Cystatin C may be a more accurate serum marker than serum creatinine, C-G, and MDRD for a prompt identification of subjects with initial reduction of renal function. An equation has been proposed to convert cys-C levels in mg/l to GFR in ml/min. This facility might contribute to increase the use of cystatin C as a sensitive marker of renal function.

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Elevated albumin: creatinine ratio may not reflect microalbuminuria in elderly female diabetic patients
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Background and Aims: Screening for microalbuminuria (MA) has been simplified by the use of albumin:creatinine ratio (ACR) as a substitute for albumin excretion rate (AER). An AER of 20 µg/min is approximately equivalent to an ACR of 2.5 mg/mmol in males and 3.5 mg/mmol in females. However, when using these sex specific definitions in a cross-sectional study, we previously found ACR in the MA range in 28% of 71 elderly female patients with AER < 20 µg/min. We therefore performed a retrospective longitudinal study of ACR and AER in older diabetic patients with persistent normoalbuminuria as determined by AER.
Materials and Methods: We randomly selected 20 females (F) and 20 males (M) from a database of 625 patients undergoing longterm followup using the following criteria: persistent absence of MA (i.e. 2 out of 3 consecutive AER measurements = 60 years at the end of followup. Serial ACR data from each participant (F 28 ± 2, M 29 ± 2) were examined using sex specific and sex independent cut offs for MA in 3 consecutive samples (table). 'False +ve' MA refers to at least one sequence of 2 out of 3 consecutive ACR results per patient in the MA range during the study.
Results: Age at baseline was F 54 ± 1, M 61 ± 1 years (p = 0.07), diabetes duration was F 11.6 ± 2.1, M 11.3 ± 2.1 years (p = 0.90), follow-up was F 11.4 ± 0.3, M 10.4 ± 0.5 years (p = 0.10), and diabetes was type 2 in F 19/20, M 14/20 (p = 0.13). During the study, a significant decline in urinary creatinine excretion was observed only in females (–1.9 ± 0.4 mmol/24 h, p = 0.04) and using sex independent cut-offs for ACR, as recommended by the American Diabetes Association, the false + ve MA rates were F 12/20, M 1/20 (p < 0.001).
Conclusion: High ACR levels without a corresponding increase in AER occur in a significant proportion of elderly female diabetic patients. This finding indicates low urinary creatinine excretion and implies a decrease in muscle mass. It may also have different renovascular implications compared with an elevated AER. In these patients, both age and sex specific cut-offs for ACR should be considered for diagnosis of MA.

	Females	Males	P
AER (µg/min) baseline	10.5 (8.1–13.9)	7.8 (5.2–9.3)	0.04
AER (µg/min) final	11.1 (10.1–13.8)	8.1 (5.8–11.6)	< 0.01
Mean urinary creatinine (mmol/24 h)	7.4 ± 0.4	13.0 ± 0.5	< 0.0001
False +ve MA *	7/20	1/20	0.04
False +ve MA **	12/20	1/20	< 0.001

AER is shown as median and interquartile range. * using sex specific cut-offs for 2 out of 3 consecutive ACR measurements (F >=3.5, M >=2.5 mg/mmole). ** using a sex independent cut-off of >=3.0 mg/mmole for 2 out of 3 consecutive ACR measurements, equivalent to >=30 µg/mg creatinine as recommended by the American Diabetes Association (2005).

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Serum erythropoetin levels in patients with type 1 diabetes mellitus

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Anaemia is a common complication of diabetes mellitus (DM) mainly in patients with diabetic nephropathy (DN) and may result in serious consequences if untreated. In DM an inadequate erythropoietin (Epo) production has been documented in anaemic patients, particularly in those with any form of albuminuria and/ or renal failure. At present, no data exists regarding serum Epo levels in type 1 DM normoalbuminuric patients with preserved renal function after more than 10 years of DM duration. The aim of this study was to determine the serum Epo levels in patients with type 1 DM with a normal urinary albumin excretion (UAE) rate and normal renal function.
Subjects and Methods: Type 1 diabetic patients with blood cell count, ferritin and renal function within the normal range with no history of diabetic complications such as nephropathy, neuropathy or proliferative retinopathy were included. In addition to routine testing, serum Epo levels were measured and other causes of anaemia or Epo deficiency were excluded. A control group of 44 healthy subjects matched for sex and age was used for comparison with a subgroup of 44 type 1 DM with a duration of DM between 10–20 years. Data is expressed as mean±SD or median (range) when considered appropriate.
Results: Seventy-seven patients were recruited [46 males, age: 30.7 ± 9.6 years; DM duration: 15.1 ± 7.0 years, BMI: 24.8 ± 3.2 kg/m^2, serum creatinine 0.9 ± 0.1 mg/dl, HbA_{1c}: 7.3 ± 1.2%, Epo: 8 (3–16) mU/ml]. Serum Epo levels were not correlated with age, HbA_{1c}, BMI, DM duration nor urinary albumin excretion rate. When the subgroup of type 1 DM patients with a DM duration of more than 10 years (13.8 ± 2.7 yrs) was compared (20 males, age 29.1 ± 7.2 years, DM duration and HbA_{1c} 7.4 ± 1.3%) with control subjects (20 males, age 31.6 ± 5,1), no differences were found in the serum Epo levels [Type 1 DM: 9.5 mU/ml (4–25) and control group: 7.9 mU/ml (4–17)].
Conclusions: Serum Epo levels are not decreased in type 1 DM with normal UAE and renal function, suggesting that serum Epo levels would decrease concurrently with the appearance of DN.

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Endothelin-1 levels are increased in patients with type 2 diabetes mellitus and diabetic nephropathy

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Background and Aims: To evaluate the relationship of plasma endothelin-1 (ET-1) levels and the urinary albumin excretion rate (UAER) in patients with type 2 diabetes mellitus (DM).
Materials and Methods: A cross-sectional study was conducted in 144 patients with type 2 DM (WHO criteria, 61 males, mean age: 58 ± 11.7 years, mean DM duration: 13.8 ± 8.2 years). UAER (immunoturbidimetry), ET-1 (ELISA), and insulin (electrochemiluminescence), were measured. Insulin resistance was estimated by HOMA index (HOMA-ir).
Results: Plasma ET-1 level had a positive correlation with 24-h UAER (r = 0.415, P <0.001), fasting plasma glucose (r = 0.311, P <0.001), A1c (r = 0.341, P <0.001), triglycerides (r = 0.310, P = 0.001), waist circumference (r = 0.259, P <0.001), BMI (r = 0.168, P = 0.02), systolic blood pressure (r = 0.294, P <0.001) and HOMA-ir (r = 0.350, P <0.001). In a linear multivariate regression analysis, ET-1 remained associated with UAER after controlling for age, gender, BMI, systolic blood pressure, A1c and total cholesterol (R = 0.521; adjusted R^2 = 0.208, P <0.001). Furthermore, there was a progressive increase in plasma ET-1 levels from patients with normoalbuminuria (n = 89, 1.18 ± 0.44 pg/ml) to microalbuminuria (n = 35, 1.47 ± 0.49 pg/ml) and macroalbuminuria (n = 20, 2.12 ± 1.12 pg/ml, P <0.001).
Conclusion: There is an independent association of ET-1 plasma levels with UAER and progressive stages of DN and this might indicate the presence of progressive endothelial dysfunction as DN progresses to more advanced stages.

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Circulating asymmetrical dimethylarginine concentrations are increased in patients with type 2 diabetes and albuminuria

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Background and Aims: Micro- and macroalbuminuria are associated with cardiovascular risk in patients with type 2 diabetes mellitus (T2DM). The competitive nitric oxide synthase inhibitor asymmetrical dimethylarginine (ADMA) which causes hypertension and cardiac dysfunction is elevated in renal failure and could promote arteriosclerosis. To evaluate the relationship between ADMA, albuminuria and cardiovascular risk we examined 87 T2DM patients.
Materials and Methods: Plasma from 34 normo-, 28 micro- and 25 macroalbuminuric patients with T2DM (age: 65 ± 1 yrs; 36 women, 51 men) who were matched for age, sex and metabolic parameters was analysed for L-arginine and ADMA. 33 patients had macrovascular disease.
Results: ADMA was significantly increased in patients with micro- and macroalbuminuria (0.63 ± 0.02 µmol/l and 0.66 ± 0.04 µmol/l, respectively) compared to those with normoalbuminuria (0.56 ± 0.02 µmol/l; p<0.05). L-arginine was comparable between all groups. Patients with micro- or macroalbuminuria and macrovascular disease had higher ADMA compared to those without macrovascular disease (0.72 ± 0.04 vs. 0.58 ± 0.02 µmol/l and 0.76 ± 0.06 vs. 0.56 ± 0.03 µmol/l, respectively; p<0.05). According to a multiple regression analysis creatinine (beta=0.40; p<0.001) and high sensitivity C-reactive protein (beta=0.29; p<0.005) were independent predictors of ADMA. Metabolic parameters were not related to ADMA.
Conclusion: Increased ADMA in T2DM patients with albuminuria is associated with renal function, subclinical inflammation and overt cardiovascular disease. The causality of ADMA for the high cardiovascular morbidity in these patients needs to be clarified in longitudinal studies.

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Nitrofurantoin, fosfomycin and cotrimoxazole in the treatment of lower urinary tract infections in type 2 diabetes patients

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Background and Aims: The treatment and prevention of the recurrence of urinary tract infections (UTI) has not been unambiguously determined up to date. There are no convincing clinical data evaluating the efficacy of various antibacterial agents in lower urinary tract infections treatment. The aim of this study was to evaluate the efficacy of nitrofurantoin, fosfomycin and cotrimoxazole in the treatment and prevention of urinary tract infections (UTI) in type 2 diabetes women.
Materials and Methods: 90 type 2 diabetes women with clinical and biological signs of UTI (with positive uroculture - isolated bacterial uropathogen sucseptible to fosfomycin, nitrofurantoin and cotrimoxazole) were enrolled into the study. All patients were 50–70 years old and have positive medical history of UTI for at least one year. Excluded criteria were: liver, kidney and haematological diseases, peripheral polyneuropathy, pregnancy and lactation. The patients were randomly divided into three groups depending on the antibacterial treatment applied: Group 1 (n=30) nitrofurantoin 0.1 g

every 12 hours before meals for 7 days, and then 0.1 g once daily at night; Group 2 (n=30) fosfomycin 3 g at 22.00 p.m. every 30 days; Group 3 (n=30) cotrimoxazole 0.48 g every 12 hours for 14 days and then 0.48 g once daily at night. The treatment was continued during 6 months and the recurrences of UTI were evaluated after next 3 months. T-student test was used for statistical analysis.
Results: After six months of long-term antibacterial treatment the therapeutic success (eradication of uropathogen and disappearance of clinical symptoms of UTI) was observed in 65% subjects in group 1, 70% in group 2 and 70% in group 3 (NS). After nine months of the study (i.e. three months after treatment discontinuation) the recurrences of UTI were stated in 60% subjects in group 3, 55% in group 1 and in 35% only in group 2 ($p<0.05$).
Conclusions: 1. Nitrofurantoin, fosfomycin and cotrimoxazole are effective agents in the treatment of UTI in type 2 diabetes patients. 2. In comparison with nitrofurantoin and cotrimoxazole, fosfomycin is the most effective agent in prevention of UTI recurrence.

PS 99
Nephropathy, experimental

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Beneficial effects of C-peptide on renal morphology in diabetic rats
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Background and Aims: To investigate the effects of C-peptide on morphological changes of diabetic nephropathy in STZ diabetic rats and on matrix synthesis and degradation in rat renal mesangial cells.
Materials and Methods: 4 groups of rats were used in non-glycemic control study: 1) normal rats (N group, n=8); 2) diabetic rats with hyperglycemia (20 mM)(HG group, n=9); 3) diabetic rats with nearly normal glycemic control (8 mM) by insulin (NG-I group, n=8); 4) diabetic rats with hyperglycemia (20 mM) treated with human C-peptide (130 nmol/kg, sc, twice daily) (HG-C group, n=9). 3 groups of rats were used in moderate glycemic control study: 1) normal rats (N group, n=11); 2) diabetic rats with moderate glycemic control (11 mM) by insulin (MG-I group, n=8); 3) diabetic rats with moderate glycemic control (11 mM) by insulin and treated with C-peptide (MG-I-C group, n=10). 8 weeks later, glomerular volume (V_{Glom}), extracellular matrix area to glomerular cross-section area ratio (A_{ECM}/A_{Glom}) and urinary albumin excretion rate (UAER) were measured. Rat mesangial cells were cultured in 3 conditions: 10 mM glucose (10 mM-G), 30 mM glucose (30 mM-G) and 30 mM glucose added with 300 nM C-peptide (30 mM-G/300nM-C). 48 hr later, type IV collagen and MMP-2 mRNA expression in mesangial cells were measured by RT-PCR. Type IV collagen protein level and MMP-2 activity in culture media were measured by ELISA.
Results: In non-glycemic control study, the V_{Glom} was $3.38 \pm 0.59 \times 10^5 \mu m^3$ in HG-C group, smaller than $6.73 \pm 1.03 \times 10^5 \mu m^3$ in HG group ($P<0.005$) and similar to $3.82 \pm 0.67 \times 10^5 \mu m^3$ in NG-I group and $3.21 \pm 0.31 \times 10^5 \mu m^3$ in N group. The A_{ECM}/A_{Glom} was 0.228 ± 0.013 in HG-C group, smaller than 0.329 ± 0.014 in HG group ($P<0.005$) and the same level as 0.246 ± 0.022 in NG-I group and 0.220 ± 0.012 in N group. The UAER of HG-C group was 334 μg/d, lower than 1028 μg/d in HG group ($P=0.059$) and higher than 72 μg/d in NG-I group and 25 μg/d in N group ($P<0.05$). In moderate glycemic control study, The V_{Glom} was $6.15 \pm 0.54 \times 10^5 \mu m^3$ in MG-I-C group, similar to $6.69 \pm 1.87 \times 10^5 \mu m^3$ in MG-I group, and larger than $4.53 \pm 1.18 \times 10^5 \mu m^3$ in N group ($P<0.05$). The A_{ECM}/A_{Glom} of MG-IC group was 0.287 ± 0.014, lower than 0.368 ± 0.042 in MG-I group ($P<0.005$) and higher than 0.228 ± 0.017 in N group ($P<0.005$). The UAER of MG-IC group was 510 μg/d, lower than 961 μg/d in MG-I group ($P<0.05$) and similar to 396 μg/d in N group. In *in vitro* study, type IV collagen mRNA expression in 30 mM-G/300nM-C culture was 34% lower than that in 30 mM-G culture ($P<0.005$) and the same level as in 10 mM-G culture. MMP-2 mRNA expression in 30 mM-G/300nM-C culture was the same level as that in 30 mM-G culture and 9% lower than that in 10 mM-G culture ($P<0.05$). Type IV collagen protein level in the media of 30 mM-G/300nM-C was 10% less than that in 30 mM-G ($P<0.05$) and the same as that in 10 mM-G. MMP-2 activity in the media of 30 mM-G/300nM-C was the same as that in 30 mM-G and 25% lower than that in 10 mM-G ($P<0.005$).
Conclusion: The mesangial expansion and glomerular hypertrophy can be prevented by 8 week C-peptide treatment in diabetic rats without glycemic control. With moderate glycemic control, C-peptide can further attenuate mesangial expansion and urinary albumin leakage than insulin alone. In mesangial cells, C-peptide can ameliorate the over expression and in turn the extracellular accumulation of type IV collagen induced by high glucose medium, but cannot prevent MMP-2 expression and activity decreases.

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Reduction of elevated urinary albumin excretion in diabetic rats by polyol pathway inhibitors correlates with changes in urinary sorbitol excretion
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Background and Aims: Elevation of urinary albumin excretion (UAE) is a sign of early renal dysfunction in human and experimental diabetes (Db). Inhibition of elevated UAE in experimental Db by polyol pathway in-

hibitors, i.e., aldose reductase inhibitors (ARIs) or a sorbitol dehydrogenase inhibitor (SDI), has been previously reported. However, the mechanism(s) of these drug effects remain(s) poorly defined. We therefore analyzed urines for UAE and polyols of normal (N) and 4-week streptozocin-diabetic rats that were untreated (UN) or treated with ARIs or an SDI.
Materials and Methods: Groups of 30 STZ-Db rats or 24 N rats consumed feed either plain or containing ARIs zopolrestat (A1) or ARI-809 (A2) at ~65 mg/kg BW/d or SDI-931 (S1) at ~2 mg/kg BW/d. At 4 weeks individual 24 hr urines were collected in metabolic cages and analyzed for UAE with Nephrat kits (Exocell, Philadelphia) and for glucose (G), sorbitol (S) (AR-metabolite of G), and fructose (F) (SDH-metabolite of S) by standard enzymatic methods. UAE data were log transformed and are given as mean (95% C.I.) (n); other data, mean±SD (n).
Results: UAE (mg/d) in UN rats was 2.0 (1.5–2.8) (24) vs. 0.7 (0.5–0.9)(24) in N rats, an elevation of 2.9-fold ($p<0.05$). A1 normalized UAE in Db rats by 69%, 1.1 (0.8–1.5) (22) ($p<0.05$ vs. UN); A2 by 81%, 0.9 (0.7–1.3) (24) ($p<0.01$); S1 by 60%, 1.2 (0.8–1.8) (24) ($p<0.05$). Urinary volume, G and F excretion were unaffected by any treatment. Urinary sorbitol excretion (USE), elevated 3.3-fold in UN vs. N, 34 ± 7 (22) vs. 10 ± 3 (24) µmole/day ($p<0.05$), was normalized by A1, 30 ± 50% (22); A2, 44 ± 32% (26), and elevated by S1, 25 ± 4-fold (22) (all $p<0.05$ vs. UN). Notably, USE correlated with UAE (p= 0.057, 0.024, _0.0011 in A1, A2, S1 groups, respectively; NS: N, UN). Excretion of F, but not S, correlated positively with food intake.
Conclusions: These data support the concept that preventing elevation of UAE in diabetic rats by an ARI or an SDI is intimately linked to inhibition of metabolic flux through the polyol pathway.

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Glucose-accelerates the transfer of touch-evoked calcium-signals in human collecting duct via an increase in connexin-43-mediated cell-to-cell communication
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Background and Aims: Aberrant sodium absorption is associated with the development of hypertension in diabetes and renal disease. In collecting duct epithelium, sodium absorption is linked to cell volume regulation and depends upon detection of osmotic and mechanical stresses. Mechano-sensitive transient receptor potential channels (TRP) and gap-junction forming connexins (Cx) are key Ca2+-dependant elements that may be important in monitoring changes in the physical environment of collecting duct, modifying absorption accordingly. The aim of the present study was to determine the role of Cx-43 mediated gap-junctions in the transfer of touch-evoked Ca2+-signals in a novel model system of the human collecting duct, under conditions of both basal and elevated glucose.
Materials and Methods: RT-PCR and western blot analysis were used to determine mRNA and protein expression for TRPV4 and Cx-43 in a human collecting duct (HCD) cell line. Imunocytochemistry was used to confirm localization of Cx-43. Mechanical stimulation was used to stimulate individual cells within a cell cluster. Fura-2-microfluorimetry and Lucifer yellow dye transfer experiments were used to assess functional parameters of cell coupling under varying glucose and calcium concentrations.
Results: Bands corresponding to TRPV4 and Cx-43 were identified from HCD mRNA and protein. Cx-43 expression was principally localized to the plasma membrane with some staining at the perinuclear region. Mechanical stimulation of an individual fura-2-loaded HCD-cell within a cell cluster evoked a transient increase in cytosolic calcium that propagated in a heptanol-sensitive manner, between coupled cells. Dye transfer between cells within a cluster was also inhibited by heptanol (1 mmol/L; n=5 separate experiments respectively). Touch-evoked changes in [Ca2+]i were still observed under Ca2+-free conditions (+EGTA;1 mmol/L), although the basal-to-peak amplitude of the response was 41% of that obtained in the presence of extracellular calcium ($P<0.05$ n=4 separate experiments). To examine the effect of elevated glucose on Cx-43 expression, HCD cells were incubated in high glucose (25 mM) for 24 and 48 hours. Cells grown under these conditions exhibited increased Cx-43 expression (234 ± 2.7% of control (5 mM) at 48 hrs; n=3, $P<0.01$). Cx-43 expression has also been linked to increased [Ca2+]i, Treatment of HCD-cells with the calcium ionophore Ionomycin (1 micro-Molar) significantly increased Cx-43 expression at 4 hrs (211.5 ± 19.9%; n=3), 6 hrs (282.7 ± 18.5%; n=3), 8 hrs (403.7 ± 25.7%; n=3), 12 hrs (266 ± 8.7%; n=3) and 24 hrs (188.5 ± 1.82%; n=3) ($P<0.01$). The velocity by which touch-evoked Ca2+-signals propagated between adjacent cells in HCD-clusters was significantly elevated following exposure to 25 mM glucose (321 ± 18.8% of control (5 mM) at 48 hrs; n=3, $P<0.05$).
Conclusion: These data confirm a functional role for Cx-43 gap-junctions in mediating the transfer of touch-evoked (TRPV4-mediated) calcium-signals between coupled cells of the human collecting duct and suggest that increases in Cx-43 expression in response to high glucose and calcium, facilitates improved signal transmission and cell-to-cell communication.

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The role of dyslipidemia and glycosaminoglycans in diabetic nephropathy pathogenesis
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Background and Aims: It has been supposed that dyslipidemia can be involved into the disturbance of glycosaminoglycan (GAG) of kidney glomerular basement membrane its composition and structure in diabetic nephropathy (DN). The goal of the study was to evaluate the interconnection between the serum level of lipoproteins and fractionary content of GAG in blood serum and urine in diabetes mellitus (DM) patients with and without DN.
Materials and Methods: 70 patients with DM type 1 (27 M, 43 F; age 40 ± 1,7), 46 patients - type 2 (16 M, 30 F; age 52 ± 2,1) and 22 healthy people were examined. 54 patients /group 1/ were normoalbuminuric, 46 /group II/ - microalbuminuric and 16 /group III/ - macroalbuminuric. The total and fractionary content of GAG in urine and blood serum and also blood serum level of high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were studied.
Results: We observed that all groups of DM patients had the decreased level of GAG blood serum total content.17,69 ± 0,94 mkg/ml in control group and 10,90 ± 0,52; 10,01 ± 0,45; 10,32 ± 0,66 in the I, II and III groups correspondingly ($p<0,00001$). Daily urine excretion of GAG total content were also decreased. The decrease of GAG content in blood serum was connected with the decrease of gyaluronic acid (GA) and chodroitin sulfate (CS) fractions, but heparan sulfate (HS) level was increased in groups I and II; 2,98 ± 0,21 ($p<0,001$), 2,58 ± 0,25 ($p<0,05$) versus controls 1,9 ± 0,1. Both daily GAG total content excretion and GA and CS were decreased. HS excretion did not differ from the controls excretion, but decreased together with DN progressing. But the HS share in total GAG content was increased in all groups of DM patients both in blood serum and urine. Positive correlation was discovered between the content of HS and LDL-C (r=0,58) and negative one with HDL-C (r=-0,62), $p<0,05$ in the blood serum and urine (r=0,53; r=-0,58) correspondingly.
Conclusion: Heparan sulfate content changings in the blood serum, increased excretion with urine and correlation among HS, HDL-C and LDL-C makes it possible to suggest LDL-C can be involved into glomerular basement membrane damage by means of GAG structure violation.

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Apoptosis and microalbuminuria in type 1 diabetic patients
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Background and Aims: Apoptosis is a model of programmed cell death. Molecules such as Fas and sFas (soluble) as well as an anti-apoptotic protein, Bcl-2, control the apoptotic process. Recent data supports that apoptosis substantially contributes to the evolution of diabetic nephropathy in type 2 diabetic patients. If microalbuminuria in type 1 diabetic patients is associated with increased renal cells apoptosis.
Materials and Methods: In 31 type 1 diabetic patients the following variables were determined: age, sex, diabetes duration, sFas, Bcl-2, HbA1c, glomerular filtration rate (GFR) and microalbuminuria, using urine albumin to urine creatinine ratio (A/C).
Results: The two groups were comparable with regard to diabetes duration and glycemic control level (17.41 years vs 14.6 and 8.1% vs 7.7 HbA1c, respectively). No statistical significance (SS) was remarked in sFas, Bcl-2 and GFR between microalbuminuric and non-microalbuminuric patients (70.71 vs 73.8 pg/ml), (65.16 vs 74.55 ng/ml) and (88.85 vs 73.43 ml/min/1.73 m^2), respectively. In microalbuminuric patients (A/C>30), there was SS between sFas and A/C ratio (r=0.736, p=0.015).Dividing patients into two subgroups [mild vs severe (A/C>150) microalbuminuric patients], SS was remarked in sFas (60.4 vs 87.2 pg/ml, p=0.047) and GFR (113 vs 69.5 ml/min/1.73 m^2, p=0.021). On the contrary, no SS was proved for Bcl-2 levels (77.96 vs 71.13).

Conclusion: In mild microalbuminuric patients sFas is positively associated with the presence of albuminuria. In non-albuminuric patients, higher levels of anti-apoptotic protein Bcl-2 were found. The above mentioned results are compatible with the current theory about diabetic nephropathy in type 1 diabetics. However, the establishment of the pathogenetic role of apoptosis in diabetic nephropathy by further investigation could provide new therapeutic tools against this very serious complication.

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Vitamin C reduces diabetes-induced apoptosis in renal proximal tubular epithelial cells

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Background and Aims: Diabetic nephropathy is a leading cause of end-stage renal disease. Previous studies have documented an increase in oxidative stress and a reduction in antioxidant status in diabetes. And several potential beneficial actions of antioxidants have been reported in the kidney in human and experimental diabetes. Oxidative stress has been suggested to play a role as a common mediator of apoptosis, and independent observations in diverse systems support a role for oxidative mechanisms in the induction of apoptosis. Recent reports provide the evidence that high ambient glucose can promote apoptosis *in vitro*, suggesting potential cellular damage by hyperglycemia in diabetic patients. However, it is uncertain whether the apoptosis occurs in the kidney during the course of diabetes. We investigated the occurrence of apoptosis in diabetic rat kidney, the role of oxidative stress and effect of antioxidant on apoptosis in diabetic rat kidney.
Materials and Methods: Male Otsuka-Long-Evans-Tokushima-Fatty rats weighing 280–320 g at 12 weeks of age were randomized into a non-treated diabetic group (n=8) and a vitamin C-treated group (n=8). Long-Evans Tokushima Otsuka rats (n=8) were used as a control. Body weight, plasma glucose concentrations, and 24-hour urine albumin levels were determined. All rats were sacrificed at 32 weeks of age. Intra-nuclear DNA fragmentation was labeled *in situ* using the apoptosis detection kit in formalin-fixed kidney tissues. Glomerular basement membrane (GBM) thickness was measured in electron photomicrographs.
Results: Urinary albumin levels in vitamin C-treated diabetic rats were significantly lower than that of untreated diabetic rats. Apoptosis was present in the epithelial cells of proximal tubules in diabetic rats. However, there were no apoptotic cells in diabetic rat glomeruli. The number of apoptotic cells was significantly decreased in vitamin C-treated diabetic rats than untreated diabetic rats. There was no significant difference in GBM thickness between two groups.
Conclusion: Vitamin C suppresses the progression of diabetic nephropathy and it may develop through the reduction of apoptosis in renal proximal tubular epithelial cells.

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Variations in antioxidant enzyme activity in patients with type 2 diabetes of African and Caucasian origin: potential association with renal disease susceptibility

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Background and Aims: Oxidative stress may be of relevance to an increased susceptibility to diabetic renal disease in patients of African origin. This consideration is supported by evidence of increased levels of plasma lipid hydroperoxide in African, compared with Caucasian patients with type 2 diabetes. Therefore, we hypothesised that these observations could be related to variations in plasma antioxidant enzyme activity.
Materials and Methods: Plasma superoxide dismutase and glutathione peroxidase activities were measured in 217 subjects. Patients with type 2 diabetes (n=75) of African and Caucasian origin were race and sex matched with healthy control subjects (n=142). Enzyme activity, cholesterol and triglycerides were respectively measured spectrophotometrically and by enzymatic methods in venous blood samples collected in the post-prandial state.
Results: Compared with healthy controls, patients with diabetes had higher superoxide dismutase and lower glutathione peroxidase activity (573.0[515.1] vs 269.6[69.6]U/l;p=0.000 and 150.4[92.9] vs 178[89.7]U/l; p=0.009) respectively. Glutathione peroxidase activity was lower, and superoxide dismutase activity higher in the African compared with Caucasian patients with diabetes (125.72 [82.26] vs 171.9 [97.49] U/l, P<0.05 and 721.86 [589.9] vs 445.33 [407.66] U/l, P<0.002). In multivariable analysis, African racial origin was an independent predictor of elevated superoxide dismutase (p=0.008) and reduced glutathione peroxidase activity (p=0.000) in patients with diabetes. Urinary albumin excretion correlated positively with glutathione peroxidase activity (rho = 0.56;p=0.002) in patients of African race.
Conclusion: African race is a determinant of superoxide dismutase and glutathione enzyme activity in the diabetic state. This pattern of antioxidant enzyme activity suggests that patients of African origin experience higher levels of renal oxidative stress compared with Caucasians and could be relevant to the differential susceptibility to nephropathy.

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Inducible overexpression of angiopoietin-1 and angiopoietin-2 in the glomeruli of adult transgenic mice via the tetracycline regulatable system

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Background and Aims: In the adult vasculature angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2), and their receptor Tie-2 retain a dual function involving angiogenesis and vascular preservation. Ang-1 regulates cellular mechanisms that mediate reciprocal interaction between endothelial cells and the surrounding matrix and periendothelial supporting cells promoting vascular network maturation. It appears that Ang-2 functions as its endogenous inhibitor promoting loosening of the vessel wall and allowing for angiogenesis and new vessel sprouting (in the presence of vascular endothelial growth factor). In the renal vasculature the balance of vascular growth intermediates is essential to the maintenance of glomerular capillary wall integrity and permeselectivity of the glomerular filtration barrier to macromolecules. Diabetes and other chronic glomerulopathies have shown an alteration in the balance of Ang-1/Ang-2 (Ang-2>Ang-1) in the kidney and glomerulus. The imbalance of the Ang-1/Ang-2/Tie2 receptor system towards an excess of Ang-2 expression present in diabetes may play an important role in the alteration of the integrity of the glomerular capillary wall where the glomerular epithelial cells, with their complex anatomical structure, play a crucial role in the regulation of macromolecules filtration. The aim of this study is to investigate the role of Ang-1/Ang-2/Tie2 receptor system on glomerular capillary integrity and proteinuria in the adult diabetic animal. To allow the expression of Ang-1 or Ang-2 without interfering on kidney and vessel development we used the tetracycline regulatable system whereby genes can be expressed with an inducible system (by administration of Doxycycline in the drinking water) in the adult animal and in our case specifically in the glomerulus.
Materials and Methods: We generated transgenic mouse lines which allow inducible overexpression of either Ang-1 or Ang-2 specifically in podocytes using a podcin promoter. We used mouse Ang-1/Ang-2 cDNA which expression was driven by a bidirectional promoter in conjunction with the β-galactosidase reporter gene. Mice were identified by standard tail DNA extraction and PCR techniques. Mice were studied when adult at 10 weeks of age. Doxycycline (2 mg/mL) was administered to the following groups (n=7) for 7 days: a) transgenic (TG) +doxycycline (DOX), b) TG -DOX, c) controls (C) +DOX and transgenic -DOX. Animals were culled and portions of kidney tissue frozen in liquid N_2 was utilized for β-galactosidase staining; for Ang-1 and Ang-2 expression analysis kidney tissue was isolated for western immunoblotting and prepared in paraffin for immunohistochemical staining.
Results: β-galactosidase studies revealed positive blue staining in TG +DOX. No detectable blue staining was identified for remaining groups, indicating an absence of leaky transgene activity. Immunohistochemistry studies showed glomerular podocyte-specific localization for both Ang-1 and Ang-2. Western immunoblottting confirmed a 2–3 fold increase (p<0.05) of Ang-1 and Ang-2 protein expression in whole kidney from TG +DOX groups when compared to the remaining 3 groups.
Conclusion: We have generated a mouse transgenic line that allows specific overexpression of angiopoietin-1 or angiopoietin-2 in the podocyte under the control of doxycycline in the adult mouse. This will allow future studies of the role of angiopoietins on the pathophysiology of diabetic glomerulopathy.
Support: EFSD/SERVIER

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Effect of a high protein diet on insulin sensitivity, leucine kinetics, and renal function in healthy elderly humans

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Background and Aims: Aging is associated with skeletal muscle wasting and decrease in muscle protein synthesis. Amino acid (AA) administration and high protein meals are reported to increase muscle protein synthesis and balance. However, AA reduce glucose uptake in tissues and cause relative insulin resistance by inhibiting early steps of insulin signaling. It has been proposed that the recommended dietary allowance of protein may not be adequate for older people. However, high protein intake could affect renal function and insulin sensitivity (SI) in this population. The present study was designed to determine the potential beneficial effect of a high protein diet on net protein accretion and adverse consequences on glomerular filtration rate (GFR) and SI.
Materials and Methods: Two groups of healthy, non-exercise-trained younger (24 ± 1y, Yng) and older (70 ± 2y, Old) men and women (Yng: 5W/5M, Old: 4W/5M) were enrolled in a randomized crossover comparison of effects of 10 days of adequate (0.9g/kgFFM/d, AP) or high (2.7 g/kgFFM/d, HP) protein diets, with a weight-maintenance calorie intake. At the end of each period, leucine flux (index of whole body protein breakdown), leucine oxidation, and non-oxidative leucine disposal (index of protein synthesis) were measured in the postabsorptive state using L-[1-^{13}C]-leucine as a tracer. GFR was determined by renal clearance of iodothalamate. SI and acute insulin response to glucose were measured with the intravenous glucose tolerance test and minimal model analysis.
Results: HP diet increased leucine oxidation in Yng and Old people ($p<0.00005$ HP vs AP in Yng and $p<0.01$ HP vs AP in Old) but the increase was significantly greater in Yng versus Old ($p<0.005$ Yng vs Old after the HP diet). Leucine flux was also enhanced by HP diet irrespective of the age of the subjects ($p<0.05$ HP vs AP diet). No age or diet effects were observed on non-oxidative leucine disposal. Plasma branched-chain AA content was increased after the HP regimen only in Yng ($p<0.05$ HP vs AP diet). Renal clearance was lower ($p<0.01$) in Old than in Yng during both diets. HP diet clearly increased GFR in Yng people (+16.9 ± 3.6%, $p<0.001$) whereas GFR decreased in Old people (-8.3 ± 6.4%, $p<0.01$). There were no changes in SI and β-cell response in either age group in response to the diets.
Conclusion: A 10-day HP diet had no beneficial effect on protein economy in elderly humans and a reduced ability to oxidize leucine was noted in the elderly following HP. The age-related high splanchnic extraction of AA may explain the lower plasma AA level in elderly after the HP diet. High intake of protein had no adverse consequence on insulin sensitivity but deleterious effect on renal function in elderly people was observed. These findings suggest that HP diets may have limited application in healthy older people.

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Inflammation and type 2 diabetes

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Increased postprandial interleukin-6 release from skeletal muscle in men with impaired glucose tolerance can be reduced by weight loss

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Background and aims: Obesity and diabetes mellitus type 2 are associated with increased levels of interleukin-6 (IL-6), a marker of inflammation. In obesity, IL-6 is produced by adipose tissue, but recently it was found that during exercise, skeletal muscle produces IL-6 as well. This study addressed the question whether IL-6 was released from skeletal muscle after a high fat meal in men with impaired glucose tolerance (IGT), a prediabetic state, and whether IL-6 release from skeletal muscle in men with IGT could be reduced by weight loss (–15% of body weight).
Materials and methods: Substrate fluxes over skeletal muscle were calculated with arterial and deep venous concentrations and blood flow over forearm muscle during fasting and after a high fat meal (61 en% fat, 33 en% carbohydrates). Forearm fluxes of soluble plasma IL-6, C-reactive protein (CRP), tumor necrosis factor alfa-receptor 1 (TNF-R1) and TNF-R2 were monitored. Measurements were repeated in IGT men after a 12-week weight loss period (4 wk VLCD, 4 wk re-introduction of the meals, 4 wk energy balance).
Results: No differences were found in BMI, body fat percentage, maximal aerobic capacity or arterial concentrations of IL-6, CRP, TNF-R1 or TNF-R1 between IGT (n = 11) and NGT (n = 9) men. Insulin sensitivity, measured during a hyperinsulinemic euglycemic clamp, tended to be lower in IGT than in NGT ($p = 0.09$), and improved after weight loss in IGT men (n = 8, $p < 0.01$). Postprandially, the increase in arterial insulin and glucose tended to be higher in IGT than in NGT, but the suppression of arterial FFA was comparable. In all subjects, it was found that IL-6 was released by skeletal muscle. No release or uptake of CRP, TNF R1 or TNF R2 was found. Muscle IL-6 release was higher in IGT than in NGT men, both during fasting (+160%, IGT = 2.26 ± 1.89 vs NGT = 0.87 ± 0.48 fmol*100 ml tissue^{-1}*min^{-1}, $p=0.04$) and after a meal (+154%, mean AUC per minute: IGT = 3.48 ± 32.63 vs NGT = 1.37 ± 0.75 fmol*100 ml tissue^{-1}*min^{-1}, $p=0.03$). Weight loss (WL) in IGT men resulted in a decrease of postprandial IL-6 release from skeletal muscle (-52%, IGT before WL = 2.99 ± 2.58, IGT after WL = 1.45 ± 1.33 fmol*100 ml tissue^{-1}*min^{-1}, $p = 0.04$), reaching levels of the obese NGT. The contribution of skeletal muscle release to systemic IL-6 was estimated, using whole body extracellular fluid estimated from bioimpedance and assumptions for total muscle mass and IL-6 half life, to be 12% (range 2% – 42%) for the whole group in the fasting state. After a meal it was estimated to be 11% (range 2% – 21%) in NGT and highest in IGT men (25%, range 2% – 66%).
Conclusions: IL-6 is released from skeletal muscle of obese, insulin resistant subjects. IL-6 release is significantly higher in IGT men during the postprandial phase when compared to obese NGT men. This indicates that a high fat load can be considered as an important metabolic stressor, evoking IL-6 release from skeletal muscle. Weight loss reduces postprandial IL-6 release, which suggests that the IL-6 release is a consequence rather than a cause of the obese, insulin resistant and/or impaired glucose tolerant state.

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Complement component 3 predicts postprandial lipemia and the metabolic syndrome

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Background and Aims: Complement component 3 (C3) is an important predictor of type 2 diabetes. We investigated whether postprandial C3-mediated fatty acid (FA) metabolism is involved in the metabolic syndrome (MetabS).

Materials and Methods: 40 MetabS+ (50 ± 8 years) and 70 MetabS- (48 ± 7 years) subjects [diagnosed based on the latest NCEP criteria]underwent a standardized 10 h oral fat load.
Results: Postprandially, MetabS+ had a higher total and incremental triglyceride response (TG-AUC: +77%; $P<0.001$ and TG-dAUC: +48%; $P<0.05$, respectively) and a higher total free fatty acid (FFA-AUC: +13%, $P<0.05$) and C3 response (C3-AUC: +26%, $P<0.001$) when compared to MetabS-. In both groups, fasting C3 was strongly associated with fasting TG, TG-AUC, TG-dAUC and insulin sensitivity (HOMA) (R=0.68, 0.67, 0.41 and 0.67, respectively, for the whole group; $P<0.001$ for each). Fasting C3 was after HDL-cholesterol the strongest determinant of TG-AUC (adjusted $R^2 = 0.54$, $\beta = 0.48$, $P < 0.001$), and showed a dose-dependent relation with the number of MetabS components and, following exclusion of the components of MetabS, it second best predicted the MetabS (adjusted $R^2=0.47$, $P<0.001$) after TG-AUC.
Conclusion: C3 is closely associated with elevated postprandial TG and FFA and the number of MetabS components, suggesting that dysfunction of adipose tissue C3-mediated FA uptake may be involved in the pathophysiology of the MetabS.

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Insulin resistance, serum adiponectin and markers of chronic inflammation in young subjects with metabolic syndrome
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Background and Aims: Metabolic syndrome is associated with an increased risk of cardiovascular diseases. Accumulating evidence strongly indicates that insulin resistance is the important pathogenic factor for metabolic syndrome. Low-grade chronic inflammation is considered to be the common background for insulin resistance and atherogenesis. The aim of the present study was to establish the relationships of The National Cholesterol Education Program (NCEP)-defined metabolic syndrome with insulin sensitivity determined with euglycemic hyperinsulinemic clamp technique, serum adiponectin and parameters of chronic inflammation in young subjects (<40 years).
Materials and Methods: The study was carried out in the group of 140 subjects (mean age 25.83 ± 5.24 years and mean BMI 27.53 ± 6.52 kg/m^2). Anthropometric and blood pressure measurements, oral glucose tolerance test (OGTT), euglycemic hyperinsulinemic clamp and estimations of serum lipids, adiponectin, C-reactive protein (hs-CRP) and interleukin 6 (IL-6) were performed in the studied group.
Results: NCEP-defined metabolic syndrome was present in 29 subjects (20.7%). Analysis of variance revealed that together with the increase of the number of NCEP criteria there was a significant decrease of insulin sensitivity ($p<0.000001$) and increase in fasting and post-load insulin concentrations (both $p<0.000001$), and an increase of hs-CRP ($p=0.000009$). The comparison of the groups according to the presence of the metabolic syndrome revealed the significantly higher fasting and post-load insulin concentrations (both $p<0.000001$), lower insulin sensitivity ($p<0.000001$), lower adiponectin ($p=0.017$) and higher hs-CRP ($p=0.000016$) and IL-6 ($p=0.019$) levels in subjects with metabolic syndrome. The significant inverse correlations between the number of NCEP criteria and insulin sensitivity ($r=-0.51$, $p<0.000001$) and adiponectin ($r=-0.30$, $p=0.007$) and positive correlations with IL-6 ($r=0.276$, $p=0.018$) and hs-CRP ($r=0.47$, $p<0.000001$) were found.
Conclusion: Our study indicates that young subjects with metabolic syndrome present, together with insulin resistance, an increased inflammatory response and lower adiponectin concentration.
The study was supported by the grant no: 3 P05A 002 25 of the Polish State Committee for Scientific Research (KBN)

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Measurement of levels of adhesion molecules ICAM-1, VCAM-1, E-selectin and lipids in type-2 diabetic subjects with proven silent ischemia and without ischemia
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Background and Aims: Cardiovascular disease (CVD) is the main cause of morbidity and mortality in subjects with diabetes. The risk markers of atherosclerosis are intensively sought. We compared levels of inflammatory markers, adhesion molecules ICAM-1, VCAM-1, E-selectin, hs-CRP and lipids of Type 2 diabetic subjects with proven silent ischemia of the myocardium and lower limbs, and without ischemia.
Material and Methods: Levels of VCAM-1, ICAM-1, E-selectin, hs-CRP, total cholesterol, LDL and HDL cholesterol, triglycerides, HbA1c, microalbuminuria, BMI and common carotid intima-media thickness (IMT) were compared within a group of type 2 diabetic subjects with proven silent ischemia of the myocardium and lower limbs (Group A) and without ischemia (Group B). Silent ischemia was proven by an exercise-myocardial SPECT and lower limbs.
Results: Both groups were comparable according to age and BMI. Group A: n=19, mean age = 55.68 ± 7.56 years, BMI=30.03 ± 4.07. Group B: n=16, mean age=56.13 ± 6.48 years BMI=30.34 ± 4.85. In the course of the Mann-Whitney U test, proof of statistically significant differences was gained only for a difference of the carotid IMT, which was greater in Group A (IMT 1.09 ± 0.28 mm vs. 0.60 ± 0.11 mm, $p<0.05$). The value of high-sensitivity C-reactive protein in both groups was at high risk for origination of CVD according to AHA, but a statistically significant difference was not proven. (hs-CRP 3.83 ± 3.72 mg/l vs. 3.37 ± 2.81 mg/l). Also levels of the intercellular adhesion molecule -1 (ICAM-1 506.32 ± 173.70 mg/l vs. 483.75 ± 182.88 mg/l), vascular adhesion molecule-1 (VCAM-1 811.16 ± 145.65 mg/l vs. 914.50 ± 555.76 mg/l), E-selectin (62.58 ± 40.13 vs. 66.13 ± 56.35) did not attain a statistically significant difference on comparison between the groups (Mann-Whitney, $p< 0.05$). We proved the same for levels of lipids, HbA1c and microalbuminuria (MAU): total cholesterol (6.03 ± 1.63 vs. 5.53 ± 1.02 mmol/l), LDL-cholesterol (4.01 ± 1.34 vs. 3.63 ± 0.83 mmol/l), HDL-cholesterol (1.13 ± 0.26 vs. 1.25 ± 0.47 mmol/l), triglycerides (2.97 ± 1.21 vs. 2.36 ± 1.16 mmol/l), HbA1c (6.99 ± 1.86 vs. 6.76 ± 1.40%), MAU (80.07 ± 57.28 vs. 65.83 ± 95.39 mg/l). On use of the Spearman correlation analysis for comparison of levels of ICAM-1, VCAM-1 and E-selectin and other measured values in Group A with silent ischaemia we proved the correlation between levels of E-selectin and triglycerides ($p<0.05$). This correlation was not proved in Group B. Triglycerides influence the reverse cholesterol transport system. This transport system moderates lipid uptake into arterial walls and has a key function in lipid accumulation.
Conclusion: Common carotid intima-media thickness was significantly greater in the group with silent ischemia. Levels of E-selectin correlated with levels of triglycerides in type 2 diabetic subjects with silent ischemia, which documents disturbance in the reverse cholesterol transport system and progression of atherosclerosis. The insignificant differences between both groups confirm the high risk of origination of CVD in Type 2 diabetic subjects. Measurement of levels of inflammatory markers and lipids and their mutual interdependence enables timely therapeutic intervention in the case of these subjects.

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High serum high-sensitivity C reactive protein is associated with cardiac sympathetic overactivity during early morning period in patients with type 2 diabetes
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Aims: To investigate whether cardiac autonomic activities or sympathovagal balance as estimated by power spectral analysis of heart rate variation during 24 h is associated with serum concentrations of hs-CRP, a sensitive predictor for cardiovascular events, in type 2 diabetic patients with and without metabolic syndrome.
Materials and methods: We studied 104 type 2 diabetic patients. Diagnosis of metabolic syndrome (MS) was based on NCEP-ATP III criteria. According to serum hs-CRP, diabetic patients then were divided into three groups: low risk (CRP<1.0 mg/l), moderate risk (1.0≤ CRP<3.0), and high risk (CRP ≥3.0). HRV was determined automatically every 5 min over 24 h using an ambulatory Holter electrocardiographic recording. Power spectral analysis of RR intervals was performed by fast Fourier transformation. Low-frequency (LF; both sympathetic and parasympathetic activities), high-frequency (HF; a pure parasympathetic activity), and the ratio of LF to HF, an index of sympathovagal balance, were used as indices of cardiac autonomic activity.
Results: Serum concentrations of hs-CRP, IL-6, PAI-1 were higher in diabetic patient with than without MS ($P<0.0001$, $P=0.0056$, $P<0.0001$, respectively). Both 24-h mean LF and LF- to- HF ratio were also significantly higher in diabetic patient with than without MS ($P=0.0397$, $P=0.0483$, respectively). LF-to-HF ratio at 6:00 AM was significantly higher in diabetic patients with high CRP than in those with low or moderate CRP ($P<0.001$, $P<0.01$, respectively. Only UAE and hs-CRP were independent factor for LF-to-HF ratio at 6:00 AM in diabetic patients.
Conclusions: Type 2 diabetic patients with metabolic syndrome had elevated markers of inflammation and cardiac sympathetic predominance. High serum concentration of hs-CRP was associated with cardiac sympa-

thetic overactivity during early morning period in patients with type 2 diabetes.

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The effects of rosiglitazone on postprandial inflammatory response in type 2 diabetes

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Objective: We postulated that in type 2 diabetes, the postprandial phase is a pro-inflammatory state that can be modulated by PPAR-γ agonists. For this purpose, we determined the effects of rosiglitazone (8 mg/d) on postprandial leukocyte counts and pro-inflammatory cytokines (IL-6 and IL-8) in patients with type 2 diabetes.
Methods and Results: A randomized, 8-week, cross-over, placebo-controlled, double-blind clinical trial was performed in 19 patients with type 2 diabetes. Standardized 6-h oral fat-loading tests were performed after each treatment period. During placebo treatment, blood leukocytes increased to a maximum 6-h postprandially, due to significant increases in neutrophils and lymphocytes. Concomitant postprandial increases were observed for IL-6 and IL-8, the major chemokines responsible for leukocyte recruitment. Rosiglitazone reduced the incremental area under the curves (dAUCs) for IL-6 (–63%, $p<0.01$) and IL-8 (–16%, $p<0.05$). The dAUC for leukocytes decreased with 37% ($p<0.05$), due to a specific reduction of neutrophils (–39%, $p<0.05$). There were no postprandial changes in plasma MCP-1 or plasma CRP, but rosiglitazone markedly reduced fasting CRP (from 3.8 ± 1.1 to 1.5 ± 0.5 mg/l, $p<0.01$).
Conclusions: Rosiglitazone attenuated the postprandial increases of neutrophils, IL-6 and IL-8 in patients with type 2 diabetes. Since inflammation is a major force driving atherosclerosis, and man lives in a postprandial period most part of the day, a reduced inflammatory response after a meal may delay progression of atherosclerosis.
This study was financially supported by a grant from GlaxoSmithKline

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Identification of soluble CD36 in blood: a possible new marker of atherosclerosis in insulin resistant conditions

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Background and Aims: Premature atherosclerosis is the major cause of morbidity and mortality in type 2 diabetes. Macrophage CD36 serves as a scavenger receptor for oxidized LDL leading to foam cell formation, and appears to be a key proatherogenic molecule. Increased expression of CD36 has been attributed to hyperglycaemia in diabetic human macrophages, and defective macrophage insulin signalling in a mouse model of insulin resistance. Thus, measurements of CD36 may represent an important marker of accelerated atherosclerosis in insulin resistant conditions.
Materials and Methods: Soluble CD36 (sCD36) was identified in blood by immunoprecipitation and Western Blotting, and an ELISA assay was established for determination of sCD36 concentrations. sCD36 was measured in blood from 10 obese type 2 diabetic patients, 11 obese, and 10 lean control subjects.
Results: Soluble CD36 was markedly elevated in type 2 diabetic patients when compared to both lean (5-fold) and obese (3-fold) control persons. Soluble CD36 significantly correlated with fasting plasma glucose ($r = 0.69$), HbA1c ($r = 0.69$), fasting insulin ($r = 0.58$), and BMI ($r = 0.52$). Interestingly, sCD36 was inversely correlated with insulin-stimulated glucose disposal ($r = -0.67$, $p < 0.0001$).
Conclusion: In conclusion, our study for the first time demonstrates sCD36 in plasma, and in agreement with the correlation between elevated soluble CD36 in type 2 diabetes and other risk factors of accelerated atherosclerosis such as glycemic control and insulin resistance, we hypothesize that soluble CD36 represents a marker of the atherosclerotic process.
Support: NovoNordisk Foundation and Danish Research Council

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Acute glucose-induced hyperinsulinemia decreases tumour necrosis factor alpha serum concentrations in women with polycystic ovary disease

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Background and Aims: Intensive insulin therapy improves outcome in critical illness and in acute myocardial infarction, possibly by reducing inflammation. It is unclear if the anti-inflammatory effect of insulin is independent of its glucose-lowering action. We tested the influence of a glucose challenge with subsequent endogenous hyperinsulinemia on serum levels of tumour necrosis alpha (TNF-α) in insulin-resistant subjects without diabetes mellitus.
Materials and Methods: Blood was drawn on days 3 and 6 after progestin-induced withdrawal bleeding in 20 patients with polycystic ovary syndrome (PCOS, median, interquartile range: age 28, 23–34 yrs; BMI 26.3, 22–28 kg/m^2) during a 75 gram oral glucose tolerance test after an overnight fast. Insulin, glucose and TNF-α concentrations (ELISA; R&D Systems) were measured at 0, 30, 60, 90, and 120 minutes.
Results: While insulin and glucose levels increased after the oral glucose load on both study days in patients with PCOS (all $p<0.0001$), their TNF-α serum concentrations decreased significantly, both on day 3 ($p=0.005$) and on day 6 ($p=0.02$). TNF-α serum concentrations decreased already 30 minutes after oral glucose load and stayed low up to the end of the study. There was a significant relationship between the serum concentrations of insulin and those of TNF-α during the oral glucose tolerance test on both study days (day 3: β-coefficient –0.07, 95% confidence interval –0.10 to –0.03, $p=0.001$, day 6: β-coefficient –0.06, 95% confidence interval –0.11 to –0.01, $p=0.01$), but not between the serum concentrations of glucose and TNF-α ($p=0.3$ and 0.4, respectively).
Conclusion: Oral glucose intake with subsequent acute hyperinsulinemia decreases TNF-α serum concentrations in women with PCOS. These findings indicate an anti-inflammatory role of insulin, even in the presence of moderate hyperglycemia.
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Oxidative stress, DNA damage and DNA repair capacity in children with type 1 diabetes mellitus

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Background: Oxidative stress (OS) occurs in conjunction with diabetes mellitus (DM). In diabetics this results in damage to lipids, proteins as well as to DNA. The aim of our work was to study the levels of oxidative stress, the degree of DNA damage as well DNA repair in a group of children with diabetes mellitus type1 (T1DM). These results were then compared with those derived from adults with T1DM.
Methods: 20 T1DM children (8 girls, 12boys, aged 9.5–19 years, average age 13.26 ±SD 2.98) were randomly chosen from a list of those treated at the Diabetology Clinic, Department of Paediatrics, University Hospital in Plzen. Average duration of illness was 2.94 ± 2.72 years. The average HbA_{1c} was 9.50 ±SD 2.52% (DCCT). Markers of oxidative stress investigated were as follows: superoxide dismutase (SOD), glutathione peroxidase (GPx), plasma antioxidant capacity (AOC), reduced glutathione (GSH) and malondialdehyde (MDA). Breaks in DNA chains within peripheral lymphocytes were investigated using the comet assay technique and indexed as DNA oxidative damage (DNAsb). Lymphocyte capacity to repair damaged DNA (DNArc) was also assessed by means of the modified comet asssay. DNRI index expressed the relationship between DNA repair and damaged DNA such that DNRI=DNArc/DNAsb. Results were compared with a group of 11 healthy children of similar age and sex (5 girls, 6 boys, aged 9.5–19 years, average age 13.73 ±SD 3.80).
Paediatric patients' results were further compared with those of a group of 23 adult diabetic individuals without microvascular complications, and with 30 adult T1DM patients with any of the following complications: retinopathy, nephropathy and/or neuropathy.
Results: Diabetic children, compared with healthy controls had significantly lower levels of SOD and GSH. MDA and DNAsb levels however were

higher though not significantly. Compared with healthy controls, increased DNA breaks in peripheral lymphocytes in diabetic children were observed. The DNArc was significantly increased in diabetic children ($p<0.05$). Extent of DNRI values in T1DM children was wide and its average was lower than in healthy children.
Even non-optimally controlled diabetic children, when compared with adult patients, had small differences in parameters of oxidative stress – except in levels of MDA. These were significantly reduced in diabetic children compared with adults ($p<0.05$). There were a lower number of DNA breaks in children compared with adults. DNArc of peripheral lymphocytes in children was significantly greater than in both adult groups ($p<0.01$ a $p<0.001$). DNRI index was thus similarly influenced.
Conclusion: In the studied population of children and adult patients with T1DM when compared with the age-related healthy population, we have confirmed increased parameters of OS and increased DNA breaks. In all diabetic groups, DNA repair capacity was significantly increased than in healthy controls. However the younger the diabetic patient, the greater the increase in levels of DNArc was ascertained.
IGA MZ CR NR7919-3/2004 Influence of individual DNA repair capacity on the course of some non-infectious diseases.

PS 101

Gene polymorphisms, lipids and lipoproteins

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Functional analysis of two newly identified LPL variations
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Background and Aims: Through the molecular screening of the lipoprotein lipase(LPL) gene in hypertriglyceridemic members of type 2 diabetic pedigrees in our previous study, two novel variants were detected in exon6(Lys312insertion C) and in exon 8 (Thr361insertion A), and also we find there was a strong linkage disequilibrium between the Lys312insC mutation and the Asn291Ser mutation. The objectives of this study were to investigate the function of the two novel variants, and also investigate whether there is a synergetic effect between the Asn291Ser mutation and the Lys312insC mutation.
Materials and Methods: The total RNA was isolated from the human adipose tissue. According to the reported cDNA sequence of the LPL, primers were designed and synthesized. By means of RT-PCR, the LPL cDNA was obtained. The LPL cDNA was cloned into pcDNA3.1Zeo (+) vector. The recombinant pcDNA3.1Zeo (+) /LPL cDNA was identified by endonucleases, PCR and DNA sequencing. Mutant LPL cDNAs with the desired mutations were generated from the pcDNA3.1Zeo (+)-wild type LPL cDNA using the Site-directed Kit (Takara,Japan). COS-1 cells were transfecteed with the recombinant LPL gene plasmid using LIPOFECTAMINE 2000™ (LF2000, Gibco BRL, Life Technologies, USA). The LPL activity in cells and the culture medium were determined by spectrophotometry.
Results: **1.** Mutant pcDNA3.1Zeo (+) LPL cDNA plasmids with the mutations Asn291Ser, Lys312insC, Thr361insA and Asn291Ser+Lys312insC were generated and transformed into COS-1 cells. **2.** LPL enzyme activity in both culture medium and cell lysate were measured. COS-1 cells and pcDNA3.1Zeo (+) plasmid themselves expressed no LPL. The COS-1 cells expressed LPL when transfected with wild type LPL cDNA. Two novel mutations transfected COS-1 cells produced decreased LPL activities in both cell lysates and medium, and when compared with the activity in wild type LPL cDNA group, there is a significant difference between the LPL activity in wild type LPL cDNA group and in mutant type LPL cDNA group. **3.** The LPL activities in cell lysates and medium of the Asn291Ser were about 60% of the wild type, and the LPL activities in cell lysates and medium of the Lys312insC were about 12% of the wild type, but the LPL activities were undetectable in cell lysates and medium of the Asn291Ser+Lys312insC transfected cells. So there was a synergetic effect between the Asn291Ser mutation and the Lys312insC mutation.
Conclusion: The two novel mutations affected the activity of the LPL to various degrees, and there was a synergetic effect between the Asn291Ser mutation and Lys312insC mutation.

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Influence of gene polymorphisms and long-term glycaemic control on lipid levels in patients with metabolic syndrome and type 2 diabetes mellitus
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Background and Aims: Dyslipidaemia of metabolic syndrome is considered to be one of the most important risk factors for atherosclerosis. It is characterised by hypertriglyceridaemia, low concentration of plasma HDL-cholesterol, predominance of small dense LDL particles and an increased concentration of plasma apoB, major protein component of LDL, IDL and VLDL particles. The pathogenesis of this type of dyslipidaemia is partially explained, but its genetic background is still unknown. Genotype may influence the lipoprotein levels by multiple mechanisms, including possible gene-environment interactions. Since poor glycaemic control was described to impair already existing diabetic dyslipidaemia, the identification of an interaction between the genotype and glycaemic control could be of great importance.

Aims: To evaluate the influence of *CETP TaqIB, LPL PvuII*, *LPL HindIII*, *LIPC -250 A/G* and *APOC3 SstI* gene polymorphisms on lipid levels in patients with metabolic syndrome and type 2 diabetes. The above mentioned polymorphisms were chosen for the present study for following reasons: cholesterol ester transfer protein (CETP) plays a key role in the transport of cholesterol-esters and triglycerides between lipoprotein particles, lipoprotein lipase (LPL) and its noncompetitive inhibitor apoproteinCIII (apoCIII) have key roles in the metabolism of triglyceride-rich particles, and hepatic lipase (LIPC gene) has an important role in the metabolism of several lipoprotein particles. A further aim was to examine possible interaction between genotype and longterm glycaemic control.
Materials and Methods: 162 patients with metabolic syndrome were included. 96% of patients had Type 2 diabetes. The patients did not take any lipid lowering treatment. The exclusion criterion was the presence of any disease that could affect lipid levels, such as thyroid disorder, liver disease, nephrotic proteinuria or renal failure. Gene polymorphisms were determined using polymerase chain reaction and RFLP-method, according to standard procedures individual for each one polymorphism.
Results: The genotype subgroups of patients divided according to examined polymorphisms did not differ in plasma lipid levels with the exception of apoB. The apoB level was significantly higher in patients with S1S1 genotype of *APOC3 SstI* polymorphism (S1S1: 1.13 ± 0.29 g/l vs. S1S2: 0.98 ± 0.20 g/l, p = 0.04) and H-H- genotype of *LPL HindIII* polymorpism (H-H-: 1.35 ± 0.39 g/l vs. H-H+ a H+H+: 1.08 ± 0.26 g/l, p = 0.01). Examining these polymorphisms we have observed also genotype-specific increase in apoB levels associated with worse glycaemic control (S1S2 genotype of *APOC3 SstI* polymorphism: r = 0.54, p = 0.018. H-H- genotype of *LPL HindIII* polymorphism: r = 0.80, p = 0.057). In the multivariate analysis the interaction of genotype and glycated haemoglobin (as an index of longterm glycaemic control) was significant independent predictor of apoB levels in *LPL HindIII* polymorphism (r^2=0.09).
Conclusion: The present study focused on patients with metabolic syndrome and type 2 diabetes showed that *LPL HindIII* and *APOC3 SstI* gene polymorphisms in association with worse glycaemic control influence negatively the plasma levels of apoB – an important predictor of atherosclerosis development.

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Associations between serum lipids, homocysteine and B vitamin levels are related to C667T MTHFR gene polymorphism in patients with type 2 diabetes

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Background and Aims: Elevated homocysteine levels and elevated serum lipids are the main coronary artery disease risk factors. There is evidence that plasma homocysteine is associated with endothelial dysfunction and insulin resistance syndrome. The aim of this study was to determine the potential relationships between serum homocysteine and lipid levels in type 2 diabetic patients.
Materials and Methods: Material included patients with type 2 diabetes consecutively recruited from diabetic outpatient clinic. Blood was taken after an overnight fast. Serum lipid levels were determined using enzymatic methods, homocysteine (HC) by Elisa, using BioRad reagents, plasma folates and vitamin B12 levels by radioimmunoassay. Polymorphism of methylenetetrahydrofoliate reductase (MTHFR) gene C667T (thermolabile variant) was determined by PCR-RFLP method.
Results: We examined 62 patients, aged 61,3+/–8.1 yrs, with mean BMI 30.7+/–4.4 kg/m^2, and mean glycated hemoglobin values of 7.9+/–1.5%. There were 30 patients with CC genotype, 27 with CT and 5 with TT genotype. Mean values of total cholesterol, HDL-cholesterol (HDL-C), LDL-C and triglycerides (TG) were as follows: 6.0+/–1.2, 1.2+/–0.4, 3.9+/–1.1 and 1.9+/–1.0. The mean concentrations of plasma folates and vitamin B12 were within normal limits. Means plasma homocysteine level was 14.3+/–4.48 µmol/l.
In the whole group of patients no significant associations between serum lipids and homocysteine levels were observed, however in patients with CC genotype significant correlation between serum TG and HC (r=0.48, p =0.007) was found. In this group of patients HC level correlated with WHR (r=0.25, p<0.01). In both group of patients with CC and CT genotype plasma folate levels correlated with BMI (r=-0.42, p=0.05). Serum homocysteine correlated with folates only in patients with BMI>30 kg/m^2 (r=–0.27, p<0.05).
Conclusion: The results of this study indicate, that in patients with CC genotype there are significant associations between plasma homocysteine and metabolic syndrome components, TG and WHR. These results suggest the role of body fat associations between serum lipids and homocysteine metabolism, what may be relevant in cardiovascular disease prevention in patients with type 2 diabetes.

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Pulse pressure correlates in patients with type 2 diabetes are related to C667T MTHFR gene polymorphism

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Background and Aims: Elevated pulse pressure (PP, the difference between systolic and diastolic blood pressure) is an important predictor of future coronary events. Recently it has been suggested that PP is powerful independent determinant of cardiovascular events in subjects with risk factors as well as in the general population.
Elevated homocysteine levels is associated with endothelial dysfunction and insulin resistance syndrome. The aim of this study was to determine the potential relationships between pulse pressure, serum homocysteine and lipid levels in type 2 diabetic patients.
Materials and Methods: Material included patients with type 2 diabetes consecutively recruited from diabetic outpatient clinic. Blood pressure was measured in the sitting position, after at least five minutes rest, the mean from two consecutive measurements was calculated.
Serum lipid levels were determined using enzymatic methods, homocysteine (HC) by Elisa, using BioRad reagents, folates and vitamin B12 levels by radioimmunoassay. Polymorphism of methylenetetrahydrofoliate reductase (MTHFR) gene C667T (thermolabile variant) was determined by PCR-RFLP method.
Results: We examined 140 patients, aged 61,3+/-8.1 yrs, with mean diabetes duration of 12.1+/–6.9 yrs and mean glycated hemoglobin values of 7.9+/–1.5%. Among them C667T MTHFR polymorphism was determined in 62 patients: there were 30 patients with CC genotype, 27 with CT and 5 with TT. Mean values of total cholesterol, HDL-cholesterol (HDL-C), LDL-C and triglycerides (TG) were as follows: 6.0+/–1.2, 1.2+/–0.4, 3.9+/–1.1 and 1.9+/–1.0 mmol/l. The mean concentrations of plasma folates and vitamin B12 were within normal limits. Mean plasma homocysteine level was 14.3+/–4.48 µmol/l. In the whole group of patients significant associations between PP and, as expected, age (r=0.36, <0.0001), systolic blood pressure (r=0.87, p<0.0001), diabetes duration (r=0.24, p<0.005), and fasting glucose levels (r=0.23, p <0.01) were observed. There were no significant differences in age, diabetes duration, plasma homocysteine, lipid and blood pressure values between patients with CC and CT genotype. In patients with CC genotype, apart from association with fasting glucose and systolic blood pressure, significant correlation between PP and total cholesterol (r=0.44, p<0.02) and LDL-cholesterol (r=0.45, p <0.02) were found.
Conclusion: The results of this study indicate, that in patients with CC genotype of MTHFR gene significant associations between pulse pressure and plasma LDL-cholesterol were found, what could be of importance in arterial stiffening prevention in this group of patients.

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CETP Taq1B genotype frecuencies in type 2 diabetic patients with hypercholesterolemia and its influence on the lipids levels before and after atorvastatin therapy

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Background and Aims: Cholesteryl ester transfer protein (CETP) plays a key role in the metabolism of HDL cholesterol; it mediates transfer of cholesteryl esters from HDL to apo B-containing lipoproteins in exchange for tryglicerides. A polymorphism in the CETP gene (Taq1B) has been shown to affect plasma CETP activity, HDL concentration, atherosclerosis progression and response to statins.We analysed in a group of type 2 diabetic patients with hypercholesterolemia the Taq 1B genotypes and alleles frequencies and its influence on the lipid profile before and after atorvastatin therapy.

Materials and Methods: 80 patients with type 2diabetes and non-treated hypercholesterolemia (LDL>130 mg/dl) were included in the study. They all were treated with atorvastatin for three months. Serum lipids were measured at the entry of the study and after three months of treatment with 20 mg/day of atorvastatin. Polymerase chain reaction (PCR),restriction fragment lenght polymorphism (RFLP), and agarose gel electrophoresis were used to determine the Taq1B polymorphism.
Results: The Taq1B genotypes frecuencies were: B1B1, 55%; B1B2, 36%; and B2B2, 9%. B1 allele, 72.5% and B2 allele, 27.5%. The individuals with at least one B2 allele (n=32) had higher levels of HDL-C (P=0.030) and apo A1 (P=0.049) than those with B1B1 genotype. Treatment with atorvastatin decreased significantly plasma levels of total cholesterol (P<0.001), LDL-C (P<0.001), tryglicerides (P=0.028) and Apo B (P<0.001) in all patients. B1B1 subjects had a greater decrease in LDL-C (P=0.032) but a lesser decrease in tryglicerides (P=0.028) than those with B1B2 or B2B2 genotypes.
Conclusion: The Taq1B polymorphism has an influence on the lipid profile and its response to statin therapy. Subjects with B1B1 genotype have a more atherogenic lipid profile (lower levels of HDL-C) and show a different response to atorvastatin treatment (greater decrease in LDL-C but lesser decrease in tryglicerides) than those with at least one B2 allele.
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Improvements in cardiovascular risk factors accompanied sustained effects on glycemia and weight reduction in patients with type 2 diabetes treated with exenatide for 82 weeks
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Background and Aims: The incretin mimetic exenatide is being investigated for its antihyperglycemic actions in patients with type 2 diabetes. In clinical trials, exenatide improved glycemic control and reduced body weight. In the current analysis, the effects of long-term administration of exenatide on cardiovascular risk factors were assessed.
Materials and Methods: 977 subjects with type 2 diabetes treated with exenatide 10 µg BID on a background of metformin, or sulfonylurea, or both have been followed in an ongoing, open-label extension of Phase 3 clinical trials. A cohort of 265 subjects (56 ± 10 y, BMI 34 ± 6 kg/m^2, baseline HbA_{1c} 8.3 ± 1.0%; mean±SD) that have completed 82 weeks of exenatide treatment have shown sustained mean (±SE) reductions in HbA_{1c} (–1.2%± 0.1) and weight (–4.6 ± 0.3 kg). The impact of exenatide treatment on lipid measures and blood pressure was evaluated.
Results:

Parameter	Baseline (±SE)	Mean Change From Baseline (±SE)	95% Confidence Interval (CI)
Triglycerides (TGs) (mg/dL)	239.17 (11.14)	–36.94 (9.66)	–55.96 to –17.91
Total cholesterol (TC) (mg/dL)	185.92 (2.41)	–2.52 (1.99)	–6.43 to +1.39
HDL (mg/dL)	37.97 (0.56)	+4.46 (0.41)	+3.64 to +5.27
LDL (mg/dL)	115.07 (2.19)	-1.41 (1.82)	-4.99 to +2.16
Apo B (mg/dL)	91.59 (1.54)	-1.30 (1.26)	-3.79 to +1.19
Systolic blood pressure (mmHg)	128.59 (0.84)	–1.48 (1.01)	–3.46 to +0.51
Diastolic blood pressure (mmHg)	78.66 (0.48)	–3.24 (0.58)	–4.37 to –2.10

Additional analysis of the cohort was done by weight change quartiles (means [kg]: –11.9, –5.2, –2.3, +1.2). In the quartile with the greatest weight reduction, the following changes in parameters were observed: TGs (–91.7 mg/dL), TC (-3.6 mg/dL), HDL (+7.4 mg/dL), LDL (+1.1 mg/dL), Apo B (–4.0 mg/dL), systolic BP (–3.3 mmHg) and diastolic BP (–4.4 mmHg). Adverse events, predominantly gastrointestinal in nature, were consistent with those observed during placebo-controlled trials.
Conclusion: Long-term administration of exenatide resulted in sustained improvements in glycemic control, progressive weight reduction, and clinically meaningful improvements in lipids and blood pressure. In general, improvements in cardiovascular risk factors were greatest in patients who experienced the greatest reductions in weight.

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Insulin glargine improves serum lipid profiles in type 2 diabetes: an observational study of everyday practice
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Background and aims: Dyslipidemia, particularly increased triglycerides (TG) and low HDL-cholesterol (HDL-C) are not only frequent, but also represent an important risk factor for macrovascular complications associated with type 2 diabetes (T2DM). Little is known about the effects of insulin glargine on serum lipid profiles.
Material and methods: In this 16-week, uncontrolled, observational study, insulin glargine was initiated in 209 patients (mean age: 55.4 ± 10.6 years; BMI: 23.4 ± 2.9 kg/m^2; duration of diabetes: 10.8 ± 6.8 years [±SD]) with type 2 diabetes requiring basal insulin for glycemic control. Decisions related to the doses, other ancillary treatments, and follow-up interval were made at the physicians' discretion. Efficacy outcomes included changes in HbA1c, fasting blood glucose (FBG), body weight, and serum lipid profile.
Results: After 16 weeks' treatment with insulin glargine, HbA1c decreased from 8.97 ± 1.85% to 7.83 ± 1.35% (n=201, p<0.0001). Dose of insulin glargine increased from 21.5 ± 8.2 IU (range 8 to 50) at baseline to 26 ± 9.7 IU (range 4 to 70) at last visit. FBG also decreased from 194.6 ± 69.2 mg/dL to 137.4 ± 47.0 mg/dL (n=126, p<0.0001). Body weight remained unchanged (mean change 0.07 ± 2.76 kg, n=203, p=0.7009). Overall serum lipid profiles were improved after 16 weeks' treatment with insulin glargine (Table). Such favorable changes were still observed even after exclusion of the patients taking statins or any other lipid-lowering drug.

	N	Baseline	Last visit	Change	P^*
All					
Total Cholesterol (mg/dL)	199	202.9 ± 48.8	187.0 ± 32.1	–15.9 ± 39.1	<0.0001
Triglyceride (mg/dL)	189	191.3 ± 127.3	165.0 ± 116.5	–27.3 ± 118.9	0.0019
HDL-cholesterol (mg/dL)	185	46.1 ± 10.5	48.1 ± 10.4	2.0 ± 7.6	0.0005
LDL-cholesterol (mg/dL)	160	119.2 ± 42.2	106.7 ± 28.3	–12.4 ± 35.5	<0.0001
Patients without statins or any other lipid lowering drugs					
Total Cholesterol (mg/dL)	142	192.4 ± 39.1	184.3 ± 31.8	-8.5 ± 30.7	0.0012
Triglyceride (mg/dL)	132	173.0 ± 100.7	155.1 ± 76.0	-20.4 ± 66.3	0.0006
HDL-cholesterol (mg/dL)	134	46.5 ± 9.9	48.4 ± 10.5	1.9 ± 7.6	0.0049
LDL-cholesterol (mg/dL)	115	112.7 ± 32.8	105.3 ± 28.9	-6.6 ± 26.0	0.0078

Values are presented as mean±SD, *H_1: $\mu_{change} \neq 0$ (paired t-test)

Conclusion: These data suggest that treatment with insulin glargine has favorable effects on lipid metabolism along with significant improvement of glycemic status. The beneficial effects of insulin glargine on the atherogenic dyslipidemia may contribute to reduce cardiovascular disease risk, among patients with type 2 diabetes.
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Value of adding fasting glucose to a lipid profile assessment for the prediction of CHD events: Whitehall II study
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Background and Aims: Current cardiovascular prevention guidelines are based on the calculated 10-year risk of a coronary (CHD) event. However, the required lab information is often incomplete in primary care, and physicians generally base the decision to complete the assessment on the presence of non-invasive risk factors. When a patient is sent for a fasting venepuncture, a glucose measurement may easily be added to the lipid pro-

file assessment at low opportunity cost. However, it is unclear if this additional information improves the CHD risk estimation.
Materials and Methods: We compared the value of adding a lipid profile or a lipid profile plus fasting glucose to a non-invasive prognostic model, using 173 incident CHD events observed during a 10 year follow-up of 7109 participants of the Whitehall II cohort study without CHD or diabetes at baseline.
Results: The ability to predict CHD events using a basic model (age >50 years and presence of hypertension) was significantly improved by adding serum total cholesterol and HDL cholesterol (areas under ROC curves 0.63 [95%CI: 0.59–0.67] and 0.75 [0.71–0.78] respectively). Addition of fasting glucose did not improve this model further (Area under ROC 0.75 [0.72–0.78]). A model based on all relevant non-invasive risk factors available in primary care (sex, age, systolic blood pressure, use of antihypertensive medication, family history of diabetes or CVD, BMI, smoking) predicted CHD events better than the basic model (Area under ROC 0.70 [0.66–0.74]). Adding total cholesterol and HDL improved this model significantly; however, further addition of fasting glucose did not (areas under ROC 0.77 [0.73–0.80] and 0.77 [0.74–0.80] respectively). Undiagnosed diabetes was identified in 72 participants (1.0%), WHO-defined impaired fasting glucose (IFG) in 244 (3.4%) and ADA-defined IFG in 1064 (15.0%).
Conclusions: Our analyses show that opportunistically adding a fasting glucose measurement to a lipid profile assessment may detect undiagnosed diabetes but does not improve the prediction of CHD events.
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Elevated plasma phospholipid transfer protein activity is a determinant of carotid intima-media thickness in type 2 diabetes mellitus
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Background and Aims: Plasma phospholipid transfer protein (PLTP) has multiple roles in lipoprotein metabolism. Experimental studies show that PLTP overexpression promotes atherosclerosis *in vivo*. Since plasma PLTP activity may be increased in Type 2 diabetes mellitus, a condition in which cardiovascular risk is strongly elevated, we tested whether PLTP activity is a determinant of carotid artery intima-media thickness (IMT), an established surrogate marker of atherosclerosis.
Materials and Methods: IMT (automatic measurement in common carotid artery by ultrasonography), clinical variables, plasma PLTP activity (phospholipid vesicles-HDL system) and lipid levels were measured in 87 non-smoking men and women with Type 2 diabetes mellitus (no insulin treatment) and 83 healthy subjects without cardiovascular disease.
Results: In diabetic patients, IMT ($p = 0.03$), pulse pressure ($p < 0.01$), plasma PLTP activity ($p < 0.001$) and plasma triglycerides ($p < 0.02$) were higher, whereas HDL cholesterol was lower ($p < 0.001$) compared to healthy subjects. In the combined groups of diabetic patients and healthy subjects, multiple linear regression analysis demonstrated that age ($p < 0.001$), male gender ($p < 0.001$) and pulse pressure ($p < 0.001$) were positive determinants of IMT. In addition, IMT was positively associated with plasma PLTP activity ($p = 0.02$) and plasma triglycerides ($p = 0.03$), and negatively with HDL cholesterol ($p = 0.03$) when included separately in the model. In diabetic patients, IMT was positively determined by plasma PLTP activity ($p = 0.03$), independent from age, gender, pulse pressure, HDL cholesterol and plasma triglycerides. Both in all subjects together and in diabetic patients separately, the effect of variance in plasma PLTP activity on IMT was as least as large as that of HDL cholesterol and plasma triglycerides.
Conclusion: This cross-sectional study shows that plasma PLTP activity is a positive determinant of IMT, suggesting that high plasma PLTP activity may contribute to accelerated atherosclerosis in Type 2 diabetes mellitus.
Support: Dutch Diabetes Research Foundation (grant 2001.00.012)

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Treatment of dyslipidemia: guidelines and clinical trials

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Case-based analysis of treatment guideline adherence in the management of patients with type 2 diabetes: the AUDIT study
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Background and Aims: The Analysis and Understanding of Diabetes and Dyslipidemia: Improving Treatment (AUDIT) study was a web-based, cross-sectional survey of 2043 diabetes specialists from 50 countries that investigated clinical practice patterns in the evaluation and treatment of dyslipidemia in type 2 diabetes mellitus (T2DM).
Materials and Methods: The AUDIT study included questions on the management of 2 typical patients with T2DM: case 1, a 52-year-old woman with no history of cardiovascular disease (low-density lipoprotein [LDL-C] 3.6 mmol/L [140 mg/dL], triglycerides 2.9 mmol/L [260 mg/dL]); and case 2, a 67-year-old man with previous myocardial infarction (MI; LDL-C 2.9 mmol/L [114 mg/dL], triglycerides 2.0 mmol/L [180 mg/dL]).
Results: For case 1, only 79% of physicians stated that they would prescribe lipid-lowering therapy, of whom 66% stated that they would use a statin and 32% a fibrate. ADA and NCEP guidelines were said to be followed by significantly more physicians who would treat case 1 with medication (30% and 20%) than those who would not (22% and 9%); significantly more of those who would not treat case 1 reported following other national society guidelines (48% vs 30%). Those who would treat case 1 reported significantly higher rates of lipid goal attainment than those who would not, for both LDL-C (56% vs 49%) and triglycerides (54% vs 49%). Significantly more physicians who would prescribe a statin for case 1 reported following NCEP guidelines than those who would prescribe a fibrate (24% vs 14%), and significantly fewer reported following other national society guidelines (28% vs 35%). Of those who cited statin prescription, significantly more patients were reported to be at target level for LDL-C (57% vs 53%) than those who cited fibrate prescription. Financial constraints to patient access were reported as the most common barrier to lipid goal attainment by significantly more physicians who would prescribe a fibrate than those who would prescribe a statin (36% vs 29%). For case 2, 83% of physicians stated that they would prescribe lipid-lowering therapy. Trends for treatment guidelines followed were similar to those for case 1. Few physicians stated that they would use a fibrate for case 2 (7%, compared with 92% statin); however, significantly more reported financial constraints as the most common barrier to lipid goal attainment than those who stated that they would use a statin (45% vs 32%).
Conclusion: Despite international guideline recommendations, patients with T2DM appear to be undertreated, with 48% of all physicians reporting that they would not prescribe statin therapy for case 1, despite an LDL-C of 3.6 mmol/L (140 mg/dL), and 24% of all physicians stating that they would not prescribe statin therapy for case 2, despite previous MI. This may be the result of inappropriate implementation of treatment guidelines by physicians and financial restrictions on statins in some regions.
Support for the conduct of the study was provided by Pfizer Inc.

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Achievement of new NCEP ATP III LDL-C and non-HDL-C goals in hypertriglyceridemic patients receiving rosuvastatin and comparator statins in the MERCURY I, MERCURY II, and STELLAR trials
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Background and Aims: Non-HDL-C targets are a secondary goal of therapy in patients with triglycerides (TG) >/=200 mg/dL (hyperTG). A recent update to NCEP ATP III goals for patients with hyperlipidemia recommends an optional LDL-C level <70 mg/dL and non-HDL-C level <100 mg/dL for very-high-risk patients; <100 and <130 mg/dL for high-risk and moderately high (medium-high)-risk patients; <130 and <160 for moderate (medium-low)-risk patients; and <160 and <190 for low-risk patients, respectively. The current study, a post hoc analysis of 2251

hyperTG patients from 3 trials with over 7000 patients comparing rosuvastatin (RSV) with atorvastatin (ATV), simvastatin (SIM), and pravastatin (PRA), assessed the efficacy of RSV to bring these patients to updated NCEP ATP III goals.
Materials and Methods: Combined LDL-C and non-HDL-C goal attainment was assessed for hyperTG subpopulations of patients randomized to RSV 10, 20 or 40 mg, ATV 10, 20, 40 or 80 mg, SIM 10, 20, 40, or 80 mg, or PRA 10, 20 or 40 mg in the following 3 trials: MERCURY I (4522IL/0081); MERCURY II (4522IL/0068) and STELLAR (4522IL/0065). Logistic regression (LR) analyses were applied to comparisons among dose groups for each study.
Results: Table shows risk categories for hyperTG population.

Risk Category	Study (weeks) STELLAR (6) n (%)	MERCURY I (8) n (%)	MERCURY II (8) n (%)
Low	248 (32.1)	21 (2.7)	0
Moderate (medium low)	124 (16.0)	32 (4.1)	0
Moderately high (medium high)	128 (16.6)	89 (11.3)	0
High (but not very high)	117 (15.1)	242 (30.8)	300 (43.4)
Very high	156 (20.2)	401 (51.1)	392 (56.6)
Total	773	785	692

Risk category not recorded for 1 patient in MERCURY I trial.

Table shows % of patients reaching LDL-C and non-HDL-C target goals.

MERCURY I	**RSV 10 (n=146)**	**ATV 10 (n=132)**	**ATV 20 (n=229)**	**SIM 20 (n=156)**	**PRA 40 (n=121)**
n (%) reaching target*	57 (39.0)	29 (22.0)**	73 (31.9)	31 (19.9)**	15 (12.4)**
MERCURY II	**RSV 20** (n=145)	**ATV 10** (n=139)	**ATV 20** (n=132)	**SIM 20** (n=139)	**SIM 40** (n=137)
n (%) reaching target	68 (46.9)	15 (10.8)	31 (23.5)	12 (8.6)	26 (19.0)
STELLAR		**RSV 10**	**RSV 20**	**RSV 40**	**RSV 80**
RSV groups		(n=51)	(n=56)	(n=60)	(NA)
n (%) reaching target		29 (56.9)	40 (71.4)	43 (71.7)	NA
ATV groups		**ATV 10** (n=51)	**ATV 20** (n=52)	**ATV 40** (n=56)	**ATV 80** (n=60)
n (%) reaching target		20 (39.2)	22 (42.3)§	39 (69.6)	40 (66.7)
SIM groups		**SIM 10** (n=46)	**SIM 20** (n=59)	**SIM 40** (n=50)	**SIM 80** (n=52)
n (%) reaching target		12 (26.1)‡	29 (49.2)§	16 (32.0)§¶	25 (48.1)
PRA groups		**PRA 10** (n=62)	**PRA 20** (n=56)	**PRA 40** (n=62)	**PRA 80** (NA)
n (%) reaching target		6 (9.7)‡	14 (25.0)‡§	23 (37.1)‡§¶	NA

NA = not applicable
*Percentages based on the number of patients with recorded data; for MERCURY I, 1 RSV 10 mg and 1 PRA 40 mg patient had no recorded data.
**P <0.001 vs. RSV 10 mg (P <0.0125 = significant); P <0.0001 vs. RSV 20 mg (P <0.0125 = significant); ‡P <0.001 vs. RSV 10 mg (P <0.002 = significant); §P <0.001 vs. RSV 20 mg (P <0.002 = significant); ¶P <0.001 vs. RSV 40 mg (P <0.002 = significant).

Conclusion: Achieving the new, more stringent combined LDL-C and non-HDL-C goals in hypertriglyceridemic patients recommended by the updated NCEP ATP III is challenging, and RSV was generally better than equivalent or higher doses of ATV, SIM, and PRA in enabling these patients to achieve target goals.

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Long-term safety and efficacy analysis from rosuvastatin-treated diabetic patients: results from the MERCURY I Study (Measuring Effective Reductions in Cholesterol Using Rosuvastatin therapY)

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Background and aims: MERCURY I (4522IL/0081), a randomized, open-label, 16-wk trial, assessed effects of switching to low doses of rosuvastatin (RSV) from commonly used doses of other statins in high-risk patients (hypercholesterolemic adults with coronary heart disease [CHD], atherosclerosis, or type 2 diabetes). Safety data were analyzed from a long-term open-label extension (OLE) up to 3 years in which all patients (pts) were titrated to the European 1998 LDL-C goal of 3.0 mmol/L (115 mg/dL). Pts were allocated to receive RSV 10–40 mg. Primary objective: to examine the long-term safety of RSV by assessing the incidence of adverse events (AEs) and abnormal laboratory data during the 3-year OLE. Secondary objective: to determine the change from baseline in lipid parameters and the proportion of pts reaching their European LDL-C goal.
Materials and methods: Of 3140 randomized pts, 2492 entered the OLE; 665 of them had type 2 diabetes. The overall safety population included all pts who received at least 1 dose of RSV therapy in the main and/or extension studies. Data were summarized descriptively.
Results: Most of the data for the primary and secondary objectives are shown in the table. The demographic characteristics of the diabetic subpopulation (ds) were similar to those of the nondiabetic subpopulation (nds). The mean age for both, predominantly Caucasian (98%) populations was 62.4 years; about 70% of pts in both groups were hypertensive. Only body mass index (BMI) >30 was substantially different (45.1% of the ds vs. 24% of the nds). Distribution of RSV doses at the final visit was similar for both groups, with the majority (78%) receiving a final dose of 10 mg. No differences were observed in treatment-related AEs, serious adverse events (SAEs), or discontinuation due to AEs between both groups. No treatment-related fatalities were observed in either group. The most common AEs in both groups were myalgia, bronchitis, nasopharyngitis, hypertension, and arthralgia. None of the pts in the ds had an alanine transaminase (ALT) elevation >3× upper limit of normal (ULN) on 2 consecutive occasions or a creatinine kinase (CK) elevation >10× ULN, and there were no cases of rhabdomyolysis in either subpopulation. In both the ds and nds, the mean creatinine plasma concentration decreased from baseline to final visit (96.5 to 91.8 and 100.1 to 96.0 µmol/L, respectively).
Conclusions: In this long-term OLE study, RSV 10–40 mg was well tolerated in a subpopulation with type 2 diabetes. The majority of pts in both subpopulations reached the European 1998 LDL-C goal of 3.0 mmol/L (115 mg/dL) and were on RSV 10 mg at their final visit.

	Patients with type 2 diabetes (n=767)	Patients without type 2 diabetes (n=2070)
Treatment-related AE	75 (9.8%)	254 (12.3%)
Treatment-related SAE	0	8 (0.4%)
Treatment-related SAE leading to death	0	0
Treatment-related discontinuation due to AE	17 (2.2%)	72 (3.5%)
	Patients with type 2 diabetes (n=618)	**Patients without type 2 diabetes (n=1724)**
% change from baseline in LDL-C at final visit	–51.1%	–47.4%
% change from baseline in TC at final visit	–34.6%	–32.2%
% change from baseline in HDL-C at final visit	12.5%	12.0%
% reaching European LDL-C goal at final visit	92.4%	90.3%

AE, adverse event; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SAE, serious adverse event; TC, total cholesterol.

The MERCURY I study was supported by AstraZeneca

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Efficacy of rosuvastatin 40 mg versus atorvastatin 80 mg in patients with the metabolic syndrome: results from a subgroup of the POLARIS study

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Background and aims: The metabolic syndrome is a constellation of metabolic conditions that increases the risk of coronary heart disease (CHD). The efficacy of rosuvastatin (RSV) 40 mg versus atorvastatin (ATV) 80 mg was compared in a subgroup of patients with the metabolic syndrome (based on NCEP ATP III definition) from the POLARIS study (4522IL/0106), a 26-week, randomised, double-blind, parallel-group, multinational study in high-risk patients (known CHD or CHD-risk equivalent, as defined by NCEP ATP III) with hypercholesterolaemia.
Materials and methods: After a 6-week dietary lead-in period, 871 patients (45–80 years) with LDL-C ≥4.1 and <6.5 mmol/L (≥160 and <250 mg/dL) and TG <4.5 mmol/L (<400 mg/dL) were randomly assigned to treatment with RSV 20 mg or ATV 40 mg daily for 2 weeks, followed by forced titration to RSV 40 mg or ATV 80 mg, respectively, for 6 weeks. The dose was maintained for 18 weeks, or, depending on LDL-C level, the dose could be reduced or additional lipid-lowering therapy could be prescribed. The primary endpoint was the change from baseline in LDL-C at 8 weeks. Secondary endpoints included LDL-C goal (2003 European and NCEP ATP III) achievement, effects on other lipid and lipoprotein levels, and safety. Statistical comparisons between treatment groups were performed on the overall intention-to-treat (ITT) population only.
Results: In the overall ITT population, a total of 470 (53.5% RSV group, 55.8% ATV group) patients had the metabolic syndrome at baseline. The percentage changes in lipids at 8 weeks for this subgroup were similar to those observed in the overall ITT population (table). In addition, the percentages of patients with the metabolic syndrome achieving their LDL-C goal with RSV 40 mg and ATV 80 mg were 83% and 71% (2003 European goal), 85% and 73% (NCEP ATP III LDL-C <100 mg/dL goal), and 41% and 19% (NCEP ATP III optional LDL-C <70 mg/dL goal), respectively. Similar levels of goal achievement were observed in the overall ITT population, with significantly more patients achieving LDL-C goals with RSV 40 mg compared with ATV 80 mg (2003 European goal: 79% vs 69%, p<0.001; NCEP ATP III LDL-C <100 mg/dL goal: 80% vs 72%, p<0.01; NCEP ATP III optional LDL-C <70 mg/dL goal: 36% vs 18%, p<0.001). Overall, both treatments were well tolerated with no myopathy, rhabdomyolysis or acute renal failure. A similar proportion of RSV- and ATV-treated patients reported serious adverse events, withdrawals and drug-related adverse events.
Conclusions: In POLARIS, the benefits of RSV in patients with the metabolic syndrome were consistent with those observed in the overall study population. RSV 40 mg was more effective than ATV 80 mg at improving LDL-C and HDL-C levels and enabled more patients with the metabolic syndrome to achieve 2003 European and NCEP ATP III LDL-C goals after 8 weeks. Both treatments were well tolerated.

Mean % change from baseline at 8 weeks	Overall ITT† population		Patients in ITT population with the metabolic syndrome	
	RSV 40 mg (n=428)	ATV 80 mg (n=432)	RSV 40 mg (n=229)	ATV 80 mg (n=241)
LDL-C	−55.9**	-52.2	-57.9	-52.2
HDL-C	+9.6**	+4.4	+12.0	+5.3
TC	−40.4	-39.3	-42.3	-39.7
TG	−22.2	-27.0‡	-27.4	-28.2
nonHDL-C	−50.7*	-48.3	-52.3	-48.0

† Statistical analysis performed on the overall ITT population only
*p<0.01, **p<0.001 vs ATV; ‡p=0.01 vs RSV

The POLARIS study was supported by AstraZeneca

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Targeted dosing of atorvastatin achieves cholesterol targets quickly in subjects with diabetes or the metabolic syndrome (the ACTFAST studies)

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Background and Aims: Despite their increased cardiovascular risk, subjects with diabetes and metabolic syndrome (MetSyn) may not reach lipid targets due to initiation of statins at insufficient starting doses and/or by lack of subsequent uptitration. An approach based on matching the starting dose of statin to the baseline and target LDL-C values may facilitate achievement of targets and was one objective of ACTFAST.
Materials and Methods: ACTFAST 1&2 were 12-week, prospective, parallel arm, open-label trials enrolling high-risk subjects (either statin-free (SF) or statin-treated (ST)) with coronary heart disease (CHD) or CHD-equivalent or a 10-year CHD risk >20%. Subjects with LDL-C >2.6 mmol/L but ≤5.7 mmol/L and triglycerides ≤6.8 mmol/L were assigned a starting dose of atorvastatin (10–80 mg/d) based on LDL-C and status of statin use at screening. The dose was doubled, if required, after 6 weeks. The primary endpoint was the proportion of subjects reaching LDL-C <2.6 mmol/L.
Results: *Diabetes (n=1024; 97% type 2, 73% SF):* At study end, 81% of SF subjects (82%, 84%, 82% and 76% with 10, 20, 40 and 80 mg, respectively) and 60% of ST subjects (61%, 68% and 47% with 20, 40 and 80 mg, respectively) achieved LDL-C target. Excluding subjects with major protocol violations (n=837), 85% of SF and 59% of ST subjects reached LDL-C target. Interestingly, in the ST subjects, atorvastatin 20–80 mg provided additional reductions in LDL-C, TG and non-HDL cholesterol (24–42%, 7–25% and 22-42%, respectively), over the statin used at baseline. C-reactive protein (CRP) decreased by 20–37% in SF (p=0.0026 for dose-response relationship) and by an additional 15–23% in ST subjects (p=0.60 for dose-response relationship). The incidence of AST/ALT >3 times and CK >10 times the upper limit of normal were 1.1% and 0.1%, respectively.
MetSyn (including those with diabetes, n=1251; 67% SF): At study end, 78% of SF subjects (81%, 84%, 82% and 66% with 10, 20, 40 and 80 mg, respectively) and 57% of ST subjects (58%, 70% and 47% with 20, 40 and 80 mg, respectively) achieved LDL-C target. Excluding subjects with major protocol violations (n=1006), 81% of SF and 58% of ST subjects reached LDL-C target. Interestingly, in the ST subjects, atorvastatin 20–80 mg provided additional reductions in LDL-C, TG and non-HDL cholesterol (22–41%, 13–32% and 21-40%, respectively), over the statin used at baseline. CRP decreased by 23–33% in SF (p=0.014 for dose-response relationship) and by an additional 21–31% in ST subjects (p=0.72 for dose-response relationship). The incidence of AST/ALT >3 times and CK >10 times the upper limit of normal were 0.9% and 0.08%, respectively.
Conclusion: ACTFAST confirmed that the use of a targeted starting dose of atorvastatin allows the vast majority of subjects with type 2 diabetes or MetSyn to achieve their LDL-C target safely with the initial dose or with a single titration. A dose-dependent decrease in CRP was observed in SF subjects.
The study was funded by Pfizer.

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Ezetimibe/simvastatin versus atorvastatin for patients who have diabetes mellitus and hypercholesterolemia

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The VYVA (VYtorin Vs Atorvastatin) trial was a multicenter, randomized, double-blind, 6-week study that compared the lipid-modifying efficacy and safety of the combination of ezetimibe/simvastatin vs. atorvastatin at milligram-equivalent statin doses (10, 20, 40, 80 mg) in 1902 randomized patients with hypercholesterolemia. This study demonstrated that ezetimibe/simvastatin provided significantly greater decreases in LDL-C and TC, and greater increases in HDL-C averaged across dose ranges. Since patients with diabetes mellitus (DM) often experience difficulty achieving therapeutic goals for LDL-C and other lipids, we evaluated the effects of these two therapies on the subgroup of patients with DM. In the VYVA trial, prevalence of DM was 221/951 (23.2%) and 207/951 (21.8%) patients treated with ezetimibe/simvastatin and atorvastatin, respectively. Percent changes averaged across the dose ranges

in LDL-C, HDL-C, TC, and TG in the DM subgroup (MITT patients) are summarized in the table below.

Parameter (mg/dL)	Ezetimibe/simvastatin N=212	Atorvastatin N=201
LDL-C		
Baseline	165.4	164.5
% change	–56.2	–45.6
Tx Diff	–10.6 (–13.4, –7.8)	
HDL-C		
Baseline	47.1	46.4
% change	6.4	3.9
Tx Diff	2.5 (0.0, 5.1)	
TC		
Baseline	252.1	251.0
% change	–40.7	–34.3
Tx Diff	–6.4 (–8.5, –4.3)	
TG		
Baseline	178.5	177.0
% change	–26.7	–25.5
Tx Diff	–1.1 (–5.5, 3.5)	

Treatment difference presented with 95% CI

Interaction of treatment and DM status was not statistically significant for any of the parameters measured. Individual mg equivalent statin dose comparisons were consistent with averaged dose data. Conclusions: DM patients in the VYVA trial treated with ezetimibe/simvastatin showed greater improvements in LDL-C, TC, and HDL-C with similar improvement in TG compared with those treated with atorvastatin. These findings were consistent with the main study results.

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Effect of ezetimibe/simvastatin versus simvastatin monotherapy on the lipid profile of hypercholesterolemic patients with metabolic syndrome

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Background and Aims: Metabolic syndrome (MetS) patients are at increased risk for developing type 2 diabetes and premature cardiovascular disease due to the presence of lipid and non-lipid risk factors. The importance of aggressively lowering LDL-C in hypercholesterolemic MetS patients has been recognized by the National Cholesterol Education Program. We evaluated the lipid-modifying efficacy of the ezetimibe/simvastatin (EZE/SIMVA) combination tablet vs SIMVA monotherapy in hypercholesterolemic patients with or without MetS.
Materials and Methods: This was a subgroup analysis of pooled data from 3 similarly designed, randomized, double-blind, placebo (Pbo)-controlled studies. After a 6- to 8-wk washout and 4-wk diet/Pbo run-in, 3083 patients with LDL-C 3.8 - 6.5 mmol/L and triglycerides (TG) ≤ 1.7–3.9 mmol/L were randomized in equal numbers to: EZE/SIMVA (10/10, 10/20, 10/40 or 10/80 mg); SIMVA alone (10, 20, 40 or 80 mg); EZE 10 mg; or Pbo for 12 wks. The primary endpoint was percent change from baseline in LDL-C for pooled EZE/SIMVA vs pooled SIMVA alone. Patients were identified as having MetS if they met 3 or more of the following; BMI ≥30 kg/m^2 (surrogate for waist circumference); TG ≥1.7 mmol/L; HDL-C <1.0 mmol/L (men); <1.3 mmol/L (women); hypertension or blood pressure ≥130/≥85 mmHg; diagnosed diabetes or fasting glucose ≥ 6.1 mmol/L.
Results: Of 2985 evaluable patients, 918 (31%) were classified as having MetS (366 on EZE/SIMVA, 384 on SIMVA, 87 on EZ 10 mg, and 81 on placebo). The baseline LDL-C value was 4.6 mmol/L for both the MetS and Non-MetS subgroups. For all efficacy endpoints, the treatment effects in MetS and Non-MetS patients were similar and consistent with the combined cohort. Pooled EZE/SIMVA significantly reduced LDL-C, non-HDL-C, apolipoprotein B (Apo B), TG, and C-reactive protein (CRP) and increased HDL-C compared with pooled SIMVA alone (table).
Conclusion: EZE/SIMVA significantly improved the lipid and inflammatory profile of hypercholesterolemic patients with MetS and thus offers an efficacious treatment option for this patient population.

	Least Squares Mean (SE) % Change from Baseline				
Parameter	**MetS Pooled SIMVA**	**Pooled EZE/SIMVA**	**Non-MetS Pooled SIMVA**	**Pooled EZE/SIMVA**	**P-value for Pooled Comparison**
LDL-C	–39.1 (0.7)	–52.0 (0.7)	–37.7 (0.5)	–52.6 (0.5)	P<0.001
Non-HDL-C	–35.5 (0.7)	–47.5 (0.7)	–34.4 (0.5)	–48.3 (0.5)	P<0.001
TG[1]	–22.7 (1.3)	–30.1 (1.3)	–16.7 (1.1)	–24.4 (0.9)	P<0.001
Apo B	–31.3 (0.7)	–41.2 (0.7)	–29.9 (0.5)	–41.8 (0.5)	P<0.001
HDL-C	8.5 (0.6)	10.5 (0.6)	6.5 (0.4)	7.3 (0.4)	P<0.05
CRP[1]	–16.7 (3.2)	–36.3 (2.7)	–14.3 (2.6)	–30.0 (2.3)	P<0.001

[1] median (SE for the median)

Study sponsored by Merck & Co. Inc.

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A comparison of effects of pioglitazone and rosiglitazone on serum lipoproteins and their subfractions in patients with type 2 diabetes and dyslipidemia

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Background and aims: By reducing insulin resistance in type 2 diabetes (T2D), pioglitazone HCl (PIO) and rosiglitazone maleate (ROSI) can affect glucose and lipoprotein metabolism. We directly compared the effects of maximal dose of PIO and ROSI on serum lipoproteins, their subfractions and size, and serum apoproteins in patients with T2D.
Materials and methods: This prospective, randomized, double blind, multicenter, multinational trial involved patients with T2D and fasting triglycerides (TG) ≥ 150 & <600 mg/dL and fasting low-density lipoprotein cholesterol (LDL-C) levels <130 mg/dl. Previous therapy included either diet alone (25%) or one oral antidiabetic agent (not PIO or ROSI). No lipid-altering agents were used prior to or during the study. After 4 weeks of placebo, patients were randomized to PIO (n=400, 30 mg QD for 12 weeks followed by 45 mg QD for an additional 12 weeks) or ROSI (n=402, 4 mg QD followed by 4 mg BID for the same intervals) therapy. Fasting serum lipoproteins were measured at a central laboratory by NMR technique. Change from baseline to the last observed value was compared between treatments using least-square means.

Results:

	PIO (n=333)		ROSI (n=325)		Difference p-value
	Baseline Mean±SD	Change Mean±SEM	Baseline Mean±SD	Change Mean±SEM	
VLDL-TG	190 ± 111	-32 ± 5	173 ± 88	22 ± 5	< 0.001
Small VLDL-TG	15 ± 10	1.5 ± 0.6	15 ± 10	1.7 ± 0.6	0.5
Intermediate VLDL-TG	75 ± 47	-2.1 ± 2.5	73 ± 42	15 ± 2.5	< 0.001
Large VLDL-TG	101 ± 96	-30 ± 4.4	86 ± 75	3.5 ± 4.4	< 0.001
VLDL size nm	57.7 ± 9.7	-5.13 ± 0.47	56.1 ± 9.4	-3.55 ± 0.47	0.008
IDL-LDL-C	4.2 ± 6.0	-0.8 ± 0.4	3.7 ± 5.5	1.7 ± 0.4	< 0.001
LDL-C	108 ± 24	3.8 ± 1.4	107 ± 25	15 ± 1.4	< 0.001
Small LDL-C	53 ± 39	-13 ± 2.1	50 ± 40	-4.8 ± 2.1	0.003
intermediate LDL-C	30 ± 29	-0.8 ± 1.8	31 ± 28	2.0 ± 1.8	0.2
large LDL-C	21 ± 24	18 ± 1.8	22 ± 25	16 ± 1.8	0.3
LDL size nm	20.0 ± 0.77	0.46 ± 0.04	20.1 ± 0.75	0.33 ± 0.04	0.006
HDL-C	40 ± 11	2.4 ± 0.4	40 ± 11	-0.1 ± 0.4	< 0.001
Small HDL-C	21 ± 5.3	-1.7 ± 0.3	20 ± 4.9	-3.8 ± 0.3	< 0.001
Intermediate HDL-C	70 ± 55	24 ± 3.1	74 ± 61	57 ± 3.1	< 0.001
Large HDL-C	13 ± 8	1.8 ± 0.4	13 ± 8	-2.0 ± 0.4	< 0.001
HDL size nm	8.68 ± 0.32	0.05 ± 0.02	8.70 ± 0.30	-0.07 ± 0.02	< 0.001
Apo B (g/L)	1.05 ± 0.20	-0.00 ± 0.01	1.04 ± 0.19	0.11 ± 0.01	< 0.001
Apo A-I (g/L)	1.14 ± 0.23	0.02 ± 0.01	1.14 ± 0.22	-0.06 ± 0.01	< 0.001
Apo C-III(g/L)	0.19 ± 0.07	-0.02 ± 0.00	0.18 ± 0.07	0.01 ± 0.00	< 0.001

VLDL, very low-density lipoprotein; IDL, intermediate density lipoproteins; HDL, high-density lipoprotein; Apo, apoprotein.

Conclusions: Although PIO and ROSI therapy exerted comparable glycemic and insulin resistance effects (data not shown), they differed in their effects on serum lipoproteins, their subfractions and size and serum apoproteins in patients with T2D. In particular, compared with ROSI, PIO decreased VLDL-TG, especially large VLDL-TG, and VLDL particle size more; reduced small LDL-C and increased LDL particle size more; and increased large HDL-C and HDL particle size more. Compared with ROSI, PIO decreased apo B more, increased apo A-I more, and reduced apo C-III more.

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Cardiac complications: genes and risk factors

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The functional effects of naturally occurring *KNCJ11* mutations associated with neonatal diabetes on cloned cardiac K_{ATP} channels

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Background and Aims: ATP-sensitive K^+ (K_{ATP}) channels link cell metabolism to electrical excitability. They are found in endocrine cells, heart, brain and skeletal muscle and play important roles in insulin secretion, neuronal excitability, cardiac stress and the response to cardiac and cerebral ischemia. K_{ATP} channels consist of pore-forming Kir6.2 and regulatory sulphonylurea receptor (SUR) subunits. The SUR1 isoform is found in β-cells and SUR2A in cardiac myocytes. Metabolic regulation of channel activity is achieved by changes in the intracellular concentrations of adenine nucleotides. Binding of ATP to Kir6.2 inhibits, whereas interaction of MgATP with SUR activates, K_{ATP} channels.
Heterozygous (*het*) mutations in Kir6.2 (*KCNJ11*) cause permanent neonatal diabetes either alone (PNDM) or in association with developmental delay, muscle weakness, and epilepsy (DEND syndrome). All mutations are gain-of-function mutations that reduce Kir6.2/SUR1 channel inhibition by ATP and increase whole-cell Kir6.2/SUR1 currents. In beta-cells, this is expected to cause membrane hyperpolarisation and thereby inhibit insulin secretion. Interestingly, children with *KCNJ11* mutations do not display obvious cardiac symptoms (likewise mice with gain-of-function mutations in Kir6.2 have few cardiac symptoms). We therefore examined the effect of one PNDM mutation (R201H) and one DEND syndrome mutation (Q52R) on the cardiac type of K_{ATP} channel (Kir6.2/SUR2A).
Materials and Methods: K_{ATP} currents were recorded from *Xenopus laevis* oocytes 1-3 days after injection with wild-type (*wt*) or mutant Kir6.2 and SUR2A mRNA. To simulate the *het* state a 1:1 mixture of *wt* and mutant Kir6.2 mRNA was used. Currents were recorded from inside-out patches with the patch-clamp technique. The pipette solution contained (mM): 140 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$, 10 HEPES (pH 7.4) plus various ATP concentrations. The Mg-free internal (bath) solution contained (mM): 107 KCl, 1 K_2SO_4, 10 EGTA, 10 HEPES (pH 7.2). The Mg-containing internal solution consisted of Mg-free solution plus 2 mM $MgCl_2$ and MgATP (instead of ATP).
Results: Both mutations reduced ATP inhibition in the absence of Mg^{2+}: IC_{50} were 21 ± 2 μM (n=12) and 35 ± 4 μM (n=9) for *het*Q52R and *het*R201H channels, respectively, compared to a reported IC_{50} of 5 μM for *wt* channels. In the presence of 2 mM Mg^{2+}, this effect was slightly greater: IC_{50} were 44 ± 9 μM (n=5) and 46 ± 4 μM (n=5) for *het*Q52R and *het*R201H channels, respectively. These compare with reported IC_{50} values of 575 μM and 141 μM for *het*Q52R and *het*R201H channels containing SUR1. K_{ATP} currents in 1 mM MgATP were 7.4 ± 1% (n=5) for *het*Q52R and 8.2 ± 0.7% (n=5) for *het*R201H channels. Importantly, these values were significantly lower than those reported for SUR1-containing channels, which were 40% for *het*Q52R and 17% for *het*R201H channels.
Conclusion: Both Q52R and R201H Kir6.2 mutations showed greatly reduced sensitivity to MgATP when coexpressed with the cardiac rather than the beta-cell isoform of SUR (SUR2A vs SUR1). Possible molecular mechanisms include less activation of SUR2A than SUR1 by MgATP, or impaired functional coupling between SUR2A and Kir6.2 mutant channels. The small K_{ATP} currents observed at physiologically relevant MgATP concentrations may account for the lack of obvious cardiac symptoms in patients carrying PNDM and DEND syndrome mutations.

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The K121Q polymorphism of the ENPP1/PC-1 gene is associated with earlier onset of insulin resistant-atherogenic phenotypes including type 2 diabetes and myocardial infarction

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Background and Aims: Insulin resistance (IR) is pathogenic for type 2 diabetes (T2D) and coronary disease (CAD). The K121Q polymorphism of ENPP1/PC-1 gene is associated with IR. Our aim was to investigate the role of the 121Q variant on the risk of T2D and CAD.
Materials and Methods: To this end, non- diabetic controls (n=638), T2D patients without CAD (n=535) and T2D patients with CAD (n=434), from Italy and the US, were studied
Results: The proportion of 121Q carriers progressively increased in the 3 groups (27.4%, 28.8% and 33.2%, respectively; adjusted p value for trend=0.045). Among diabetic subjects (n=969), 121Q carriers had increased risk to have developed T2D before the age of 65 years (adjusted OR, 95% C.I.= 2.26, 1.26–4.03, p=0.006) and to have had MI (n=156) within the age of 50 years (adjusted OR, 95% C.I.=3.17, 1.46–6.88, p=0.007). The 121Q variant was also associated with an increased risk of CAD (adjusted OR, 95% C.I.=1.47, 1.01–2.18, p=0.049) in diabetic subjects who did not smoke (n=546).
Conclusion: In conclusion, the ENPP1/PC-1 121Q variant is associated with a progressive deterioration of the IR-atherogenic phenotype from non diabetic to diabetic subjects without and with CAD. Among diabetic individuals, the 121Q variant is also associated with earlier onset of T2D and MI. This polymorphism may be therefore helpful to identify individuals at risk of early T2D and coronary disease.

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Polymorphism T45G of adiponectin gene and risk of myocardial infarction in type 2 diabetic subjects with high vascular risk: the DIABHYCAR Study

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Background and Aims: Adiponectin concentration is decreased in type 2 diabetic subjects (T2D) and in subjects with coronary artery disease. A polymorphism of the adiponectin gene, T45G, is associated with higher risk to develop insulin resistance and T2D. Our objective was to evaluate the association between the adiponectin gene T45G SNP and the risk of future myocardial infarction (MI), in a cohort of T2D subjects, followed prospectively.
Materials and Methods: Participants were 3123 French T2D with high vascular risk from the DIABHYCAR Study (non-insulin-dependent DIABetes, Hypertension, microalbuminuria or proteinuria, CArdiovascular events, and Ramipril). Eligible participants were older than 50 years, had type 2 diabetes (defined on the basis of receiving current treatment with at least one oral antidiabetic agent), and had urinary albumin excretion ≥20 mg/l, in two successive random urine samples. The principal exclusion criteria were a serum creatinine concentration >150 µmol/l; treatment with insulin, an ACE inhibitor, or an angiotensin II receptor blocker; documented congestive chronic heart failure; myocardial infarction during the past three months; urinary tract infection; and previous intolerance to an ACE inhibitor. The cardiovascular events were prospectively followed up (median duration of follow-up 47 months). Genotyping has been achieved by PCR followed by allelic hybridization with fluorescent probe.
Results: In the whole population, the G allele was associated with the incidence of MI, fatal or not (Chi^2: p = 0.003). In particular, the incidence of MI was higher in patients with GG genotype (see Table). In men, at entry, the HbA1C value of GG homozygotes (8.16 ± 0.23%) was higher than that of TT (7.64 ± 0.04%) or GT (7.76 ± 0.07%), (p=0.044). The Cox model showed that GG homozygous have higher risk for myocardial infarction (adjustment for sex, age, BMI, HbA1c, albuminuria, blood pressure, HDL-cholesterol, treated or not by Ramipril and previous myocardial infarction): RR = 3.24 [95% CI 1.40–7.49], p=0.006). No interaction between Ramipril and T45G polymorphism on the incidence of MI was detected.
Conclusion: In a cohort of type 2 diabetic subjects, followed prospectively, the T45G polymorphism of adiponectin gene was associated with an increased risk of myocardial infarction.

Table: Polymorphism T45G of the adiponectin gene and incidence of myocardial infarction (MI)

	TT	TG	GG	G frequency
No MI	2,236 (97.4%)	734 (96.2%)	58 (90.6%)	14%
Incident MI	60 (2.6%)	29 (3.8%)	6 (9.4%)	22%

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The study of relationship between PON1 activity as well as oxLDL level and coronary artery lesions in patients with coronary artery disease and diabetes mellitus

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Background and Aims: Paraoxonase 1 (PON1) and Oxidized Low Density Lipoprotein (oxLDL) are main risk factors of coronary atherosclerosis disease(CAD). Many animal experiments and case-control studies had proved that there were correlation between PON1 as well as oxLDL and CAD. But the relationship between PON1 as well as oxLDL and the characteristics of the lesions of coronary artery in patients of CAD with hyperglycemia is unclear. The aim of this study is to investigate the relationship between PON1 activity as well as serum oxLDL levels and patients diagnosed CAD via coronary arteriography with normal or hyperglycemia.
Materials and Methods: 194 CAD patients diagnosed by coronary angiography, whom were divided into the group of CAD with IFG (n=62), the group of CAD with DM (n=46), and group of CAD with normal fasting plasma glucose (n=86). 90 non CAD with normal fasting plasma glucose control subjects were determined by coronary angiography. Serum PON1 activities and oxLDL levels were measured in all the cases, the relationship between PON1 activity as well as oxLDL levels and the number of stenosed coronary arteries were evaluated.
Results: 1. PON1 activities in CAD patients are obviously lower than those in non CAD controls, and much lower in CAD with hyperglycemic patients. Meanwhile oxLDL levels in CAD and CAD with hyperglycemic patients are obviously higher than in the non CAD controls.(table 1)
2. In patients of CAD with normal fasting plasma glucose, there are no statistically significant changes in the levels of oxLDL (203.7 ± 28.25vs. 228.76 ± 31.76EU · dL-1, p=0.212) and PON1 activites (113.09 ± 88.56 vs. 107.45 ± 97.95 µkat · L-1, p=0.39) when patients of multiple stenosed vessels compared with patients of single stenosed vessel. 3. In patients of CAD with IFG, there are no statistically significant changes in PON1 activities (173.58 ± 96.86 vs.190.03 ± 101.48 µkat · L-1, p=0.314), while oxLDL levels are higher (122.58 ± 34.97 vs. 107.62 ± 26.86 EU · dL-1, p=0.039) when patients of multiple stenosed vessels compared with cases of single stenosed vessel. 4. In patients of CAD with DM, the patients of multiple stenosed vessels have lower PON1 activities than those of single stenosed vessel (178.32 ± 92.95 vs.191.08 ± 98.54 µkat·L-1, p=0.047), while oxLDL levels of the former are higher than the latter (134.37 ± 30.18 vs. 104.57 ± 29.55 EU·dL-1, p<0.001).
Conclusion: Our data suggest that PON1 activity is decreased and oxLDL level is increased in CAD patients, and these changes are much more obvious in CAD with hyperglycemia. It implies that PON1 is one of the factors avoiding the oxidation of LDL. The higher oxLDL level and lower PON1 activity may are predicting factors that multiple vessels involved in CAD with hyperglycemia.

(means±SD, * P<0.05 vs control; # P<0.05 vs CAD.

	Controls (n=90)	CAD (n=86)	CAD+IFG (n=62)	CAD+DM (n=46)	F	P
FPG (mmol·L-1)	4.65 ± 0.71	4.70 ± 0.62	6.28 ± 0.46*	11.54 ± 4.06* #	172.66	0.000
TC (mmol·L-1)	4.42 ± 1.21	4.95 ± 1.22*	5.36 ± 1.25* #	5.37 ± 2.11* #	6.166	0.000
TG (mmol·L-1)	1.70 ± 0.99	1.90 ± 0.92	2.00 ± 0.99	2.12 ± 1.25	1.599	0.190
HDL (mmol·L-1)	1.41 ± 0.38	1.18 ± 0.29*	1.18 ± 0.35*	1.18 ± 0.27*	8.670	0.000
LDL-C (mmol·L-1)	2.74 ± 0.90	2.95 ± 0.87	3.31 ± 0.99*	3.21 ± 1.01*	4.947	0.002
oxLDL (EU·dL-1)	95.49 ± 24.35	110.54 ± 29.31*	115.14 ± 28.89*	122.06 ± 29.28* #	10.935	0.000
PON1 activity (µkat·L-1,)	248.103 ± 96.82	218.56 ± 91.04*	189.34 ± 63.63* #	183.59 ± 70.33* #	4.085	0.007

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Insulin-like growth factor-1 in patients with acute myocardial infarction but without previously known type 2 diabetes – report from the GAMI-trial

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Background and Aims: Low levels of insulin-like growth factor-1 (IGF-1) have been associated with increased risk for cardiovascular disease and type 2 diabetes. This substudy from the GAMI-trial (Norhammar et al. 2002, Lancet 359:2140–2144) characterizes patients with acute myocardial infarction but without previously known type 2 diabetes regarding the IGF-1 system.
Materials and Methods: 168 patients with acute myocardial infarction were classified before discharge by means of an oral glucose tolerance test (OGTT) as having normal glucose tolerance, impaired glucose tolerance or type 2 diabetes. The term abnormal glucose regulation was defined as the presence of impaired glucose tolerance or diabetes. 185 sex- and age matched healthy persons served as controls. Fasting IGF-1, measured before hospital discharge, was related to glucose tolerance, insulin resistance and beta-cell dysfunction.
Results: Concentrations of IGF-1 were lower in patients than in controls (Median [Q1,Q3], µg/L 117.0 [75.0–145.0] vs. 122.0 [99.0–143]; p = 0.009). In patients IGF-1 successively decreased with increasing glucose intolerance (Table 1). IGF-1 at discharge remained a significant predictor of abnormal glucose regulation in a multiple logistic regression analysis both at hospital discharge and after 12 months (OR = 0.29; p = 0.020 and OR = 0.29; p = 0.034 respectively). In men IGF-1 was the strongest predictor of abnormal glucose regulation at hospital discharge and after 12 months (OR = 0.17; p = 0.006 and OR = 0.13; p = 0.017 respectively). Other predictors in the different models were fasting blood glucose, free fatty acids, proinsulin, HbA1c and triglycerides. IGF-1 was the only parameter that remained a significant predictor in all models. IGF-1 predicted beta-cell function estimated as adjusted insulinogenic index at 30 minutes during the OGTT [(Δ30I/Δ30G)/HOMA-IR] in a multiple regression model including fasting blood glucose and insulin (p = 0.037). Patients with a family history of type 2 diabetes had significantly decreased concentrations of IGF-1 (p = 0.012). There was no significant difference of insulin-like growth factor binding protein-1 (IGFBP-1) between the glucose tolerance groups.
Conclusion: The present report provides evidence for the importance of IGF-1 for glucose homeostasis. Low levels of IGF-1 at discharge predict persistent abnormal glucose regulation in patients with acute myocardial infarction already before hospital discharge and may be an ideal marker for gluco-metabolic dysfunction in such patients.

Table 1. Age-adjusted IGF-1 in patients. Values presented as geometric means and 95% CI.

	Normal glucose tolerance (n = 55)	Impaired glucose tolerance (n = 58)	Type 2 diabetes mellitus (n = 55)	p
IGF-1 (µg/L)	119.7 (106.4,134.7)	103.5 (92.4,115.9)	91.4 (81.6,102.4)	0.007

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Plasma NT-proBNP and long-term mortality in type 2 diabetes

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Background and Aims: Raised N-terminal pro brain natriuretic peptide (NT-proBNP) is associated with a poor cardiac outcome in non-diabetic populations. Elevated NT-proBNP predicts excess mortality in type 1 diabetic nephropathy and an increased incidence of major cardiovascular events in microalbuminuric type 2 diabetic patients. This study investigated the prognostic value of NT-proBNP in a large cohort of type 2 diabetic patients.
Materials and Methods: In a prospective observational follow-up study 315 type 2 diabetic patients with normoalbuminuria (n=188), microalbuminuria (n=80), and macroalbuminuria (n=47) were followed for 15.5 (0.2–17.0) years. Plasma NT-proBNP concentration was determined by immunoassay at baseline. End points were overall and cardiovascular mortality.
Results: Of the patients, 162 patients died (51%) hereof 119 (74%) due to cardiovascular causes. All cause mortality was increased in patients with NT-proBNP in the second and third tertiles (hazard ratios compared with the first tertile, 1.70 (1.08–2.67) and 5.19 (3.43–7.88) (p<0.001)). These associations persisted after adjustment for urinary albumin excretion rate, glomerular filtration rate, and cardiovascular risk factors (covariate adjusted hazard ratios 1.48 (0.93–2.36) and 2.55 (1.57–4.15) (p<0.001)). This increased mortality was attributable to more cardiovascular deaths in the second and third NT-proBNP tertile (unadjusted hazard ratios 1.63 (0.96–2.77) and 4.88 (3.01-7.91), (p<0.001); covariate adjusted 1.41 (0.82–2.44) and 2.33 (1.31-4.14) (p=0.01). NT-proBNP levels increased with urinary albumin excretion rate (p<0.001), and when patients with normo-, micro- and macroalbuminuria were analysed separately, NT-proBNP levels above the median (62 ng·l^{-1}) were consistently associated with increased overall and cardiovascular mortality in all three groups (p<0.001).
Conclusion: Elevated circulating NT-proBNP is a strong predictor of the excess overall and cardiovascular mortality independent of urinary albumin excretion rate and conventional cardiovascular risk factors in patients with type 2 diabetes.
Support: Roche Diagnostics

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Coronary calcification and novel cardiovascular risk factors in type 2 diabetes: the Prospective Evaluation of Diabetic Ischaemic Heart Disease by Coronary Tomography (PREDICT) study

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Background and Aims: Coronary Calcification Score (CACS) obtained by electron beam tomography has been shown in several prospective studies to predict coronary heart disease (CHD) events in the general population. There is limited data specific to diabetes. The PREDICT Study aims are to determine specifically in type 2 diabetes (i) the association between conventional and novel cardiovascular risk factors and CACS and (ii) the predictive value of CACS for CHD events. We have previously shown an increased calcification compared to non-diabetic data and significant correlation between CACS and wait hip ratio (WHR).

Materials and Methods: The relationship between homocysteine (Hcy), high sensitivity CRP, Homa IR index, apoproteins A1 and B, and triglyceride/HDL cholesterol ratio and CACS was studied in 573 individuals from the PREDICT diabetic cohort.
Results: In univariate analysis there were significant (p<0.001) associations between WHR, CRP, Hcy, systolic blood pressure and use of antihypertensive drugs with CACS. There was a negative association with CRP. In a multivariate model adjusting for the possible interaction of these and other factors the significant associations between CACS WHR, and age remained. Associations with Hcy and use of antihypertensive drugs were of borderline significance and the negative association with CRP remained.
Conclusion: Age, WHR and type 2 diabetes per se, and not novel cardiovascular risk factors, appear to be the major factors influencing CACS in type 2 diabetes.
Support: Tomkins and British Heart Foundations

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Cardiotrophin-1: another marker of cardiovascular disease in type 2 diabetes?
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Background and Aims: Cardiotrophin-1(CT-1) is a newly isolated cytokine that belongs to interleukin-6 cytokine superfamily. CT-1 induces cardiac myocyte hypertrophy through the JAK/STAR pathway, and promotes cardiac myocyte survival via the MEK/MAPK and PI3K/Akt pathway. The level of CT-1 is increased in the presence of coronary heart disease,myocardial infarction and genetic hypertension. There is a lot of data revealed that all diabetic patients should be considered to be coronary heart disease risk equivalent. Could CT-1 indicate cardiovascular disease of diabetes, we observed the changes of plasma CT-1 level in type 2 diabetes patients and it with coronary heart disease.
Materials and Methods: We use the Biotin-Streptavidin-enzyme-linked immunosorbent assay (BSA-ELISA) to measure the levels of plasma CT-1 in 42 patients with type 2 diabetes (T2DM), 20 patients of coronary heart disease (CHD), 45 patients of type 2 diabetes with coronary heart disease (T2DM+CHD) and 35 normal controls (NC).All coronary heart disease patients were diagnosed by coronary arteriongraphy results: An atherosclerotic lesion was considered significant when a stenosis ≥50% of the lumen in at least one major vessel was documented.
Results: The difference of plasma CT-1 levels in the four groups was significant (ANOVA, $P<0.001$). The levels of plasma CT-1 in T2DM+CHD group (465.38 ± 102.23pg/ml) was apparently higher than that of CHD group (382.59 ± 94.68 pg/ml, $P<0.05$), T2DM group (99.56 ± 21.77 pg/ml, $P<0.01$)and NC (31.46 ± 9.73pg/ml, $P<0.001$), the level in CHD group was significantly higher than that of the T2DM group ($P<0.01$) and NC ($P<0.01$), and the level in T2DM was higher than that of NC ($P<0.05$). The level of plasma CT-1 was related with blood pressure and myocardium enzyme: Creatine kinase (r= 0.795, $P<0.001$); Hydroxybutyric acid dehydrognase (r=0.647, $P<0.001$); Diastolic blood pressure (r=0.484, $P<0.001$); Systolic blood pressure (r=0.422, $P<0.001$); Lactic acid dehydrognase-1 (r=0.381, $P<0.01$); Isoenzyme of creatine kinase (r=0.345, $P<0.05$). Stepwise regression analysis revealed that Creatine kinase (CK) was the most significant agent affecting the plasma CT-1 (See table 1).
Conclusion: The elevation of plasma CT-1 in type 2 diabetes patients suggests that cardiovascular disease initiated in the presence of diabetes; Plasma CT-1 indicated the severe degree of diabetic cardiovascular disease, can be act as a marker of cardiovascular disease in type 2 diabetes. The plasma CT-1 was related with hypertension in type 2 diabetes suggested that hypertension aggravated diabetic cardiovascular disease.

Table 1: Stepwise regression analysis of the plasma CT-1 level and other parameters

Independent Variable	Unstandardized Coefficients		Standardized Coefficents	t	P
	B	Std. Error	Beta		
Constant	344.992	23.791		14.501	0.000
CK	0.344	0.042	0.795	8.185	0.000

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Impact of insulin resistance and visceral fat accumulation on cardiac function evaluated by tagged magnetic resonance imaging
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Background and Aims: Preferential abdominal fat accumulation is associated with insulin resistance and has been related to increased risk of adverse cardiovascular events. Magnetic resonance imaging (MRI) is widely used for the quantification of abdominal fat content but also allows one to characterise myocardial deformation patterns during the heart cycle. The goal of this study was to investigate the impact of visceral fat (VAT) accumulation and insulin resistance on the changes in systolic shortening (E1) using cine dynamic MRI tissue tagging.
Methods: We recruited 40 male subjects, of whom 14 had newly diagnosed, untreated essential hypertension (HT; blood pressure 154 ± 3/92 ± 2 mmHg) and 26 had confirmed normal blood pressure (NT; blood pressure 123 ± 2/70 ± 2 mmHg). All subjects received a 75g OGTT with determination of glucose, insulin and C-peptide profiles for 3 hours. Insulin sensitivity was estimated from the OGTT glucose and insulin concentrations (OGIS method). VAT and subcutaneous (SAT) fat depots (kg) was measured by multislice MRI, while cardiac function and systolic shortening (E1) were determined by cardiac MRI and the tagging approach.
Results: HT subjects had similar SAT (3.4 ± 0.3 *vs* 3.1 ± 0.2 kg, p=ns) but larger VAT than NT subjects (1.7 ± 0.2 *vs* 1.2 ± 0.1 kg, p=0.01) and were less insulin sensitive (335 ± 17 *vs* 379 ± 12 ml/min/m^2, p<0.04). VAT and insulin sensitivity were reciprocally related to one another (r=-0.45, p<0.01). All subjects had normal cardiac function and measured parameters were within previously established normal ranges. As expected, left ventricular mass was higher in the HT group than in controls (78 ± 4 *vs* 69 ± 2 g/m^2, p=0.04). On the other hand, left and right ventricular (LV and RV) end-systolic (ESV) and end-diastolic (EDV) volumes were similar between the two groups: LV-EDV = 73 ± 2 *vs* 67 ± 3 ml/m^2; LV-ESV = 26 ± 1 *vs* 22 ± 3 ml/m^2; RV-EDV = 75 ± 3 *vs* 71 ± 2 ml/m^2; RV-ESV = 29 ± 2 *vs* 26 ± 1 ml/m^2, in NT vs HT, respectively, all p=ns. Ejection fractions of both ventricles were similar (LV = 64 ± 1 *vs* 67 ± 3%; RV = 61 ± 1 *vs* 63 ± 2% in NT *vs* HT, both p=ns). In contrast, systolic shortening (E1) was reduced in HT (–5.5 ± 0.6% *vs* –7.3 ± 0.3% of NT, p=0.009). In univariate analysis, E1 was significantly related to age (r=+0.52, p=0.002), blood pressure (r=+0.46, p<0.01), BMI (r=+0.52, p<0.003), insulin sensitivity (r=-0.69, p<0.0001) and visceral fat (r=0.61, p=0.0002). In a multiple regression model, only insulin sensitivity (partial r=-0.54, p<0.002) and VAT (r=0.35, p=0.05) were significant correlates of E1.
Conclusions: Insulin resistance and preferential visceral fat accumulation are important determinants of ventricular systolic function.

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Ventriculo-arterial coupling in type 2 diabetes assessed non-invasively by tissue Doppler and a wave intensity approach

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Background and Aims: Arterial stiffness is important in the prognosis of cardiovascular (CV) disease. CV involvement in type 2 diabetes (DM) with preclinical myocardial disease still awaits clarification and remains a clinically relevant issue. In order to evaluate ventriculo-arterial coupling in preclinical CV disease, we used a wave intensity approach.
Materials and Methods: 36 DM patients (HbA1c 6.8 ± 1.2%, 84% on insulin and 33% on OAD therapy) and 36 control individuals (CO), all without evidence of hypertension and coronary artery disease were selected. Groups were comparable with regards to age (57 ± 10 years), CV risk factors, cardiac medication, left atrial and ventricular size and wall thickness. Myocardial velocities were assessed by tissue Doppler and the characteristics of pulse wave intensity noninvasively, using a combined Doppler and echotracking system (Aloka SSD-5500, Tokyo). By measuring diameter and flow in the right carotid artery, instantaneous changes were derived in local arterial stiffness (epsilon, pulse wave velocity) and in wave intensity (calculated as the product of pressure and velocity changes with respect to time). From the two characteristic wave intensity peaks resulting from forward travelling waves, the early systolic compression wave (W1) is related to LV inotropism and the late systolic expansion wave (W2) influenced by peripheral resistance.
Results: DM patients had significantly higher heart rate (68 ± 9 vs 62 ± 9 bpm, p<0.05), systolic blood pressure (135 ± 17 vs 124 ± 16 mmHg, p<0.01), rate pressure product (p<0.01), pulse pressure (p<0.02) and LV filling pressure (p<0.009). Diastolic myocardial velocity was decreased (8,0 ± 2.1 vs 10.0 ± 2.0 cm/s, p<0.0002) and so was the mitral inflow velocity (p<0.03) and the maximal flow velocity in the carotid artery (52 ± 14 vs 62 ± 13 cm/s, p<0.03). Epsilon and pulse wave velocity were higher (p<0.02) and so was W1 (10552 ± 3956 vs 7958 ± 3785, p<0.05) but not W2.
Conclusion: In patients with type 2 diabetes without evidence of CV disease, a preserved pump function is maintained against increased arterial impedance at expense of increased myocardial oxygen requirements, possibly from enhanced sympathetic drive. Facing the known limitations of myocardial perfusion and of mitochondrial energy supply in diabetes mellitus, this constellation requires therapeutical compensation. The wave intensity approach may have the potential for therapeutic monitoring.

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Early decrease in diastolic function in patients with type 1 diabetes mellitus as an initial manifestation of diabetic cardiomyopathy

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Background and Aims: Diabetic cardiomyopathy is a well-defined complication of diabetes mellitus (DM) that occurs in the absence of ischemic, vascular, and hypertensive disease. The natural history of diabetic cardiomyopathy remains unclear, mainly due to concurrent coronary disease or hypertension. Since the presence of confounding factors is less likely in young patients, they constitute a suitable study model to analyze early stages of diabetic cardiomyopathy. The aim of this study was to evaluate left ventricular diastolic dysfunction (LVDD) in young patients with type1 DM free of cardiovascular disease symptoms.
Materials and Methods: Indexes of left ventricular diastolic filling were measured by Doppler-echocardiography in 35 young patients with type 1 DM (mean age -30,1 ± 1,8 years) and 15 control subjects (mean age -29,9 ± 1,8 years) without clinical evidence of heart disease. Data presented as M±m. The results were compared using Student's paired test.
Results: No patient had chest pain or electrocardiographic changes during exercise testing. All patients had a normal ejection fraction (53,9 ± 2,3 vs 56,1 ± 4,3, (p > 0,05) in those with and without DM, respectively). A diagnosis of LVDD was made when at least one of the following parameters was present in the echocardiographic study: isovolumetric relaxing time (IRT) >100 ms, deceleration time (DT) > 220 ms or early filling rate peak/late filling rate peak ratio (E/A) < 1. According to these criterions twelve (34,2%) of the 35 diabetic patients had evidence of diastolic dysfunction as assessed by the presence of at least two abnormal variables of mitral inflow velocity. In particular, the ratio of peak early to peak late (atrial) filling velocity was significantly decreased in diabetic compared with control subjects (E/A ratio 0,9 ± 0,05 vs 1,2 ± 0,06, p< 0,05), the DT was 257,1 ± 28,2 ms vs 186 ± 14,6 ms p<0,05, in those with and without DM, respectively. Also the IRT was increased in diabetic patients compared with control subjects (130 ± 14,0 ms vs 79,3 ± 6,1 ms, p< 0,05). LVDD in diabetic patients correlated with duration of DM but did not correlate with diabetic microvascular complications (retinopathy, nephropathy) or peripheral neuropathy.
Conclusion: Our data suggest that more than one-third of young type 1 DM patients with a normal systolic ventricular function have subclinical diastolic dysfunction which serves as a marker of a diabetic cardiomyopathy.

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Augmented myocardial function is associated with optimized postprandial glucose in patients with type 2 diabetes

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Background and Aims: Hyperglycemia is an important cause of the predominantly diastolic dysfunction in patients with type 2 diabetes. The hypothesis was studied that myocardial function benefits from optimized postprandial glucose values in a comparison of three insulin treatment regimes.
Material and Methods: 56 patients with type 2 diabetes were studied by ultrasound before and two hours after a standardized breakfast (48 g carbohydrates). The insulin regimes had been unchanged for 12 months and were: 1) conventional (CT) with mixed insulin 2x/d in 17 patients, 2) intensive (ICT) with rapid analogue insulin 3x/d and NPH insulin 1x/d in 19 patients and 3) supplemental (SIT) with rapid insulin 3x/d in 20 patients. The three groups had comparable age of patients (64 ± 7 years), fasting glucose (160 ± 37 mg/dL) and lipid values, insulin dose at breakfast (19 ± 14 IE), hemodynamic and cardiovascular data and cardiac medication. Global systolic (Vs) and diastolic (Ve) myocardial velocities were assessed by tissue Doppler.
Results: HbA1c was comparable in CT, ICT and SIT (6,5 ± 0.6, 6,2 ± 0.6 und 6,4 ± 0.7%). The postprandial increase of glucose was 56 ± 48 mg/dL in CT but 6 ± 46 mg/dL in ICT (p<0.006) and 14 ± 64 mg/dL in SIT (p<0.05). Ve was significantly decreased in CT (7.0 ± 0.8 cm/s) vs 8.0 ± 1.4 in ICT (p<0.02) and 7.9 ± 1.3 cm/s in SIT (p<0.03).Vs had similar tendencies (p<0.08). There was a significant inverse correlation between the postprandial decrease of Ve and increase of glucose (p<0.013) in all patients. Postprandially, the index of LV filling pressure was significantly higher in CT (12 ± 4 mmHg) vs 9 ± 2 mmHg in ICT (p<0.006) and 9 ± 3 mmHg in SIT (p<0.02).
Conclusion: The studied insulin regimes modify postprandial more than fasting glucose values and demonstrate augmented myocardial function associated with optimized postprandial glucose values.

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Impact of anemia on left ventricular remodeling in type 2 diabetic patients with end-stage renal disease

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Background and Aims: The excess mortality in diabetic end-stage renal disease (ESRD) is mainly due to cardiovascular events. Over the last decade, the therapeutic options for the treatment of ESRD have progressively increased. In 1994, erythropoietin therapy for pre-dialysis patients was launched in Japan. The objective of this study is to evaluate factors affecting left ventricular (LV) remodeling over a 10-year period in type 2 diabetic patients with ESRD.
Materials and Methods: M-mode echocardiography was performed consecutive diabetic ESRD patients by one experienced examiner less than 2-months before the start of dialysis therapy. Two groups (group A & group B) of type 2 diabetic patients with ESRD but no overt cardiac diseases (myocardial infarction, valvular failure, hypertrophic cardiomyopathy) on echocardiography were enrolled. Group A (64 men, age (mean(SD)) 54 (12) years) was enrolled during 1990 to 1993 and group B (65 men, age 57(11)years) was enrolled during 2000 to 2003. LV remodeling was graded based on 4 categories; normal (N), concentric remodeling (CR), concentric hypertrophy (CH), and eccentric hypertrophy (EH).
Results: In terms of LV remodeling, the ratio of EH in group A (56%) was significant higher than group B (39%)(p<0.05), while N (12 vs 9%),CR(0 vs 5%),and CH (32 vs47%) were not significant. Group A had shorter known duration of diabetes(16(7) vs 18(8) years, p<0.05), higher HbA1c (7.3(1.6)

vs 6.7(1.2)%, p<0.05), elevated systolic blood pressure(164(23) vs 150(19)mmHg, p<0.001) and diastolic blood pressure (83(12) vs 77(13)mmHg, p<0.05), and lower hematocrit (24.2(5.1) vs 26.6(3.7)%, p<0.001), as compared with group B. A logistic regression analysis revealed that lower hematocrit was an independent risk factor for EH in LV remodeling (OR -0.008, CI -0.015~0.002), excluded known duration of diabetes, systolic and diastolic blood pressure, and HbA1c.
Conclusion: It is suggested that improving anemia with erythropoietin therapy reduces eccentric hypertrophy in type 2 diabetic patients with ESRD.

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Subclinical myocardial dysfunction in asymptomatic diabetic subjects. A tissue Doppler imaging study
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Background and Aims: Diabetes Mellitus (DM) substantially increases the risk of development of heart failure. Early detection of impaired left ventricular function could be very important for the prognosis in this group of patients.The objective of the present study was to investigate the left ventricular suclinical systolic and diastolic dysfunction in patients with DM using newly developed echocardiographic techniques.
Materials and Methods: The study population consisted of 49 consecutive diabetic (43 type 2 DM, 26 men, aged 53 ± 12 yrs, mean duration of DM 8 ± 6 years, 23 insulin treated) and 29 non-diabetic (non-DM) subjects (13 men, aged 53 ± 12 yrs) with no clinical heart disease. Participants underwent full echocardiographic examination including 2-dimensional, conventional Doppler as well as Tissue Doppler Imaging (TDI). Systolic (Sm), early (Em) and late diastolic (Am) longitudinal myocardial velocities (cm/s), as well as longitudinal strain (%), systolic (SSR), early diastolic (EDSR) and late diastolic strain rate (LDSR)(1/sec) were measured at 12 basal and medial myocardial segments (apical views) and averaged. For the assessment of the combined left ventricular myocardial performance, Tei index was calculated [Tei index = (Isovolumetric Contraction + Isovolumetric Relaxation Time)/Ejection Time].
Results: Subjects with DM were found to have significantly impaired systolic and diastolic left ventricular function compared with non-DM subjects. Independent predictors of average longitudinal myocardial systolic velocities of the left ventricle were glucosylated haemoglobin (beta=-0.43, p<0.001) and age (beta=-0.33, p<0.01).Left ventricular ejection fraction, systolic and diastolic TDI parameters and Tei-index in two groups are presented in the Table.
Conclusion: Diabetic patients with no clinical heart disease have impaired left ventricular function at rest. Tissue Doppler Imaging is an excellent diagnostic tool for the identification of subclinical myocardial dysfunction.

ECHO DATA

	Sm	Em	Am	strain	SSR	EDSR	LDSR	Ejection fraction	Tei
DM	3.87 ± 0.7	4.71 ± 1.4	5.32 ± 1.3	15 ± 3%	1.50 ± 0.3	1.52 ± 0.3	1.78 ± 0.4	64 ± 7%	0.51 ± 0.1
non-DM	4.53 ± 0.8	5.64 ± 1.7	4.24 ± 1.0	18 ± 3%	1.64 ± 0.3	1.65 ± 0.4	1.45 ± 0.5	66 ± 5%	0.42 ± 0.1
p value	0.001	0.001	0.001	0.001	0.054	0.076	0.003	0.091	0.002

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Measurement of microvolt T-wave alternans, a new arrhythmic risk stratification test, in type 2 diabetic patients without ischaemic heart disease
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Background and Aims: The analysis of microvolt T-wave alternans (TWA) has been recently proposed as a new, non-invasive, diagnostic tool for identification of patients with an increased risk of ventricular arrhythmias and sudden cardiac death. The prevalence of microvolt TWA positivity is very high in patients with myocardial infarction, congestive heart failure and implantable defebrillators (approximately 40–60%), but it is very low in clinically healthy individuals (approximately 2–5%). Although type 2 diabetes is strongly associated with an increased risk of ventricular arrhythmias and sudden cardiac death, currently there is a lack of available data on measurements of microvolt TWA in people with type 2 diabetes mellitus.
Materials and Methods: We enrolled 43 type 2 diabetic volunteers (M/F= 33/10, age= 64 ± 5 years, diabetes duration= 11 ± 8 years) with no known or suspected ischaemic heart disease (IHD) (as ascertained by medical history, resting electrocardiogram, Holter monitoring and conventional bicycle ergometry). Microvolt TWA analysis was performed non-invasively during a submaximal exercise with the patients sitting on a bicycle ergometer by the use of the CH 2000 system (Cambrigde Heart Inc, Bedford, MA).
Results: Microvolt TWA test was positive in 9 (21%) patients, negative in 32 (74.4%) and with indeterminate results in the remaining 2 (4.6%) patients; these latter were removed from statistical analysis. Patients with TWA positivity had markedly higher plasma HbA1c levels than those with a negative TWA test (8.1 ± 0.9 vs. 7.2 ± 0.8%, p<0.01). Plasma HDL concentrations tended to be lower but did not reach statistical significance. Age, sex, BMI, blood pressure, plasma LDL cholesterol and triglyceride levels, 24-hour heart rate variability (as calculated by Holter monitoring), QTc interval duration, smoking history, diabetes duration and treatment, and microvascular complication status (inclusive of retinopathy, sensorimotor neuropathy and nephropathy) did not differ significantly between the groups. In regression logistic analysis, plasma HbA1c concentration was the only significant predictor of TWA positivity (odds ratio 5.7, 95% C.I. 1.3–26, p=0.023) after adjustment for all potential confounders.
Conclusion: These results indicate that in type 2 diabetic patients without clinically manifest IHD, TWA positivity is highly prevalent (approximately 20%) and is closely associated with a poor glycaemic control, thus suggesting that the quantitative assessment of microvolt TWA can provide additional prognostic information for the arrhythmic risk stratification of people with type 2 diabetes. Further prospective studies are clearly needed to establish whether a more aggressive treatment of diabetes may contribute to reduce the increased risk of ventricular arrhythmias and sudden cardiac death of patients with type 2 diabetes.

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Evaluating the control of cardiovascular risk factors (CRF) in type 2 diabetic patients submitted to coronary angiography (CA): from guidelines to routine practice
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Introduction: The risk of coronary artery disease (CAD) death and serious nonfatal CAD events is markedly increased in diabetic patients (pts) relative to nondiabetic subjects. Controlling CRF is the cornerstone to prevent and/or delay the appearance of these complications.
Aim: To evaluate the prevalence and the adequacy of control of CRF according to the recommendations of the American Diabetes Association (ADA) and to associate these factors to CAD in a population of diabetic patients submitted to elective CA.
Methods: 173 consecutive pts submitted to elective CAs were evaluated. All patients underwent an interview and a clinical examination, and had their fasting plasma glucose (FPG), glycosylated haemoglobin (HbA_{1c}), cholesterol (total and fractions) and triglycerides analysed. Extension of coronary stenosis and severity scores were determined through the CAs analysis.
Results: A small proportion of pts reached the recommended ideal goals for CRF: HbA_{1c} < 7% (37.6%), FPG < 130 mg/dL (17.4%), LDL cholesterol <100 mg/dL (15%), HDL cholesterol >= 40 mg/dL for males and >= 45 mg/dL for females (29.5%), total cholesterol< 200 mg/dL (68.8%), triglycerides < 150 mg/dL (57.8%), blood pressure < 130/80 mmHg (12.7%), body mass index (BMI) <25 kg/m^2 (28.3%), waist circumference (WC) <88 cm for females and <102 cm for males (49.1%). 120 pts (69.9%) were in use of acetyl salicylic acid and 147 (85%) did not smoke or had quit at least 2 years earlier. Pts who had four or more controlled CRF presented a significant greater number of normal coronaries (58.3% vs 40.8%, p=0.038). Females had a worse control of CRF, showing less controlled factors compared to men (3.29 ± 1.17 vs 4.32 ± 1.64, p<0.001). Pts who did not have CAD showed a better control of HbA_{1c} (54.2% vs 31.2%, p=0,004). The logistic regression analysis showed that the CRF independently associated with CAD are: age (odds ratio [OR]: 1.05, 95% CI, 1.01–1.09; p=0.007), male sex (OR: 3.95; 95% CI, 1.76–8.85; p=0.001), BMI >=25 kg/m^2 (OR: 3.11; 95% CI, 1.22-7.92; p=0.017), FPG >= 130 mg/dL (OR: 3.35; 95% CI, 1.52-7.40; p=0,003) and

family history (FH) of cardiovascular disease (CVD) (OR: 2.97; 95% CI, 1.31-6.75; p=0.009). The CRF independently associated to the severity of the disease measured by the severity scores in the linear regression analysis are: BMI >= 25 kg/m^2 (Estimated Effect [EE]: 2.19; 95% CI, 0.17–3.91; p=0.013), FPG >= 130 mg/dL (EE: 2.43, 95% CI, 0.88–3.98, p=0.002), FH of CVD (EE: 1.91; 95% CI, 0.33–3.49; p=0.018).
Conclusion: The evaluated population showed a high prevalence and poor control of CRF. Establishing health policies to identify and control CRF in the diabetic population is essential to the reduction of morbidity and mortality caused by CVD.
Support: Madre Tereza Hospital

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The metabolic syndrome is associated with early mortality in acute coronary syndrome
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Background and Aims: It is well known that mortality is 2 fold increased in diabetic patients with acute myocardial infarction (AMI). In addition, blood glucose level at the admission is correlated with mortality in diabetic and non-diabetic patients with AMI. Many studies show a strong relationship between metabolic syndrome (MS) and myocardial infarction, but there is no data concerning the association between MS and early mortality after an AMI. The term «cardiovascular vulnerable patients» was recently proposed to define subjects susceptible to an acute coronary syndrome based on atherosclerotic plaque, blood coagulability and myocardial vulnerability. In this retrospective study, we evaluated in patients admitted in the Intensive Care Unit (ICU) for an acute coronary syndrome (ACS), the prevalence of MS and its association with early mortality.
Materials and Methods: Medical records of 858 patients admitted in ICU between January 2002 and December 2003 for an ACS were evaluated; 329 patients were excluded (transfer, ACS not the cause of hospitalisation, 2nd hospitalisation the same year). MS was defined according to W.H.O, except that microalbuminuria was not measured during the stay: MS = glucose intolerance (glycemia ≥7.8 mmol/l) + 2 additional components; pre-MS = glucose intolerance + 1 additional component. Early mortality, laboratory values and treatment of ACS were evaluated. Early mortality was defined as mortality during the hospital stay.
Results: Five hundred and twenty-nine patients were included. The mean age was 64 ± 13 yrs and 72% of the patients were males. One hundred and ninety presented MS (36%), 116 pre-MS (22%) and 223 no MS (42%). Forty-seven patients (9%) died during the hospital stay. MS and pre-MS were predictive factor of early mortality (OR=5.9 (95% CI 2.0–17.9) and 15.0 (95% CI 5.0–44.4), p<0.0001). In multivariate analysis matched for age, sex and CK levels, MS and pre-MS were independently associated with early mortality (p=0.01 and <0.0001). Concerning investigations and treatment of ACS: 90% of MS patients and 88% of pre-MS patients underwent coronary angiography vs. 96% of no MS patients (OR=0.6 and 0.5, p=0.006). Two or 3-vessel disease was present in 59% of MS patients and 45% of pre-MS patients vs. 45% of no MS patients (OR=1.4 and 1.0, p=0.01). A coronary bypass was performed in 13% of MS patients and in 10% of pre-MS patients vs. in 3% of no MS patients (OR=1.8 and 2.1, p=0.0004).
Conclusion: Patients with MS or pre-MS are cardiovascular vulnerable patients. MS should therefore be taken in consideration to quantify the risk of early mortality after an ACS. Coronary angiography was less performed in MS and pre-MS patients despite their high cardiovascular risk.

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Type 2 diabetes and metabolic syndrome in post MI patients
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Background and Aims: People with metabolic syndrome (MS) have a 2-3 times higher risk for coronary heart disease (CHD); coronary mortality is also higher. Components of MS predict type 2 diabetes mellitus (DM2) and may provide a common pathway linking DM2 and CHD. Diagnosis and treatment of MS in post-myocardial infarction (MI) patients has not been widely studied. We therefore examined the prevalence of MS and DM2, as well as abdominal obesity, lipid profile, fibrinogen, and C-reactive protein in 144 acute (approximately 2 mos) post MI patients.
Materials and Methods: After acquiring informed consent and medical history, all patients underwent routine anthropometric and blood pressure measurements. Blood samples were collected to assess lipid profile, fibrinogen, and C-reactive protein. Additionally, a 75 gm oral glucose tolerance test was given to all patients after a 12 hour fast.
Results: Patients had a mean age of 53.8 years (86% Hispanic; 8% African American; 74% male). Of 144 patients, 86 (60%) had DM2 (n=63) or MS without diabetes (n=23). Altogether 54 patients (38%) met NCEP criteria for MS. Among the diabetic patients 25% had not been previously diagnosed (UDx). Impaired glucose tolerance (IGT) was observed in 42 patients (28%), of whom 15 had MS. Abnormal waist circumference was seen in 60 patients (42%). Although virtually all patients were on hypolipidemic agents that influence lipid values, and abnormal readings of CRP may be expected in all patients because of the recent MI, those with IGT or UDx exhibited higher mean lipid levels than normoglycemic patients, respectively, for total serum cholesterol (166 mg/dL, 172 mg/dL vs. 152 mg/dL); triglycerides (135 mg/dL, 151 mg/dL vs. 128 mg/dL); and LDL-cholesterol (100 mg/dL, 93 mg/dL vs. 86 mg/dL). Similarly, fibrinogen (363.44 mg/dL, 392.72 mg/dL vs. 330.17 mg/dL) and C-reactive protein (6.19 mg/dL, 4.71 mg/dL vs. 3.82 mg/dL) were also higher in the IGT and UDx patients than in normoglycemics. Interestingly, the UDx's with MS comprised the highest percentage of patients with abnormal fibrinogen (71%) and CRP (100%) levels.
Conclusion: Results imply that: (1) MS occurs in about 40% of post MI patients; (2) over 70% of post MI MS occurs in glucose intolerant (DM2 and IGT) patients; and (3) that more than one third of post MI patients had undiagnosed glucose intolerance; they showed worse CHD risk factors than the normoglycemic individuals. These findings although obtained relatively early after an acute MI, indicate that it is important to look for MS in post MI patients. Identification of the MS in these patients offers an opportunity not only to tailor pharmacologic treatment for hypertension and lipid abnormalities, but to capitalize on patients new health concerns through aggressive behavioral interventions (i.e., diet and exercise) that can reduce the risk factors for DM2 and reinfarction in post MI patients.
Support: NHLBI

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Coronary artery disease in type 2 diabetes: effect of weight loss on lipid peroxide levels and antioxidant enzyme activity
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Background and Aims: Previous studies have demonstrated that weight loss might be beneficial for reducing the risk for coronary artery disease (CAD). However, the influence of the weight loss on the systemic levels of oxidative compounds and antioxidant enzyme activity, postulated to play important roles in atherogenesis, has not yet been clarified. The aim of this study was to evaluate the effect of a moderate weight reduction diet on (a) lipid peroxide levels, being important oxidative agents, (b) antioxidant glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activity and (c) other related metabolic risk factors for CAD before and after implementation of a moderate weight regimen in 26 obese Type 2 diabetic patients with CAD (group A) and in 22 obese nondiabetic patients with (group B).
Materials and Methods: In each patient, the presence of CAD was determined angiographically. The dietary regimen (individually adjusted, 1400–1800 kcal/day, 55% carbohydrates, 30% fat and 15% proteins) was applied for 6 months. Before and at the end of its application, we determined lipid peroxide levels in thiobarbituric acid-reacting substance (TBARS) assay, GSH-Px and SOD activity by spectrophotometry, together with anthropometric (body mass index (BMI) and waist to hip ratio (WHR)) and metabolic parameters (insulin resistance (IR) by homeostasis model assessment (HOMA), plasma insulin (PI) by RIA, total cholesterol (Ch), HDL-Ch, LDL-Ch and triglyceride (TG) levels by chromatography).
Results: After the implementation of the dietary regimen, we found a small but not significant decrease in the TBARS levels, while both GSH-Px and SOD activity significantly increased in both groups (A: GSH-Px: 29.3+/-2.7 vs 24.6+/-3.6 U/gHb; SOD: 9.7+/-1.1 vs 7.2+/-1.3 U/mgHb, p<0.05; B: GSH-Px: 33.2+/-4.8 vs 27.6+/-3.7 U/gHb; SOD: 12.3+/-1.9 vs 8.7+/-1.4 U/mgHb, p<0.05). Simultaneously, we detected the decrease in BMI (A: 26.7+/-0.7 vs 29.6+/-1.1 kg/m2, p<0.05; B: 25.4+/-0.4 vs 28.9+/-0.8 kg/m2, p<0.05), together with a decrease in WHR (A: 0.92+/-0.02 vs 0.98+/-0.02, p<0.05; B: 0.92+/-0.01 vs 0.97+/-0.01, p<0.05), HOMA levels (A: 6.6+/-0.7 vs 11.7+/-2.0, p<0.01; B: 2.4+/-0.2 vs 4.9+/-0.6, p<0.01), PI (A: 13.2+/-0.7 vs 25.7+/-3.4 mU/l, p<0.01; B: 11.7+/-0.7 vs 18.4+/-2.8 mU/l, p<0.05), total Ch (A: 5.9+/-0.3 vs 6.9+/-0.5 mmol/l, p<0.05; B: 6.1+/-0.3 vs 7.0+/-0.5 mmol/l, p<0.05), LDL-Ch (A: 3.9+/-0.3 vs 4.6+/-0.3 mmol/l, p<0.05; B: 4.0+/-0.2 vs 4.9+/-0.2 mmol/l, p<0.05) and TG (A: 1.7+/-0.1 vs 3.1+/-0.4 mmol/l, p<0.05;

B: 1.4+/-0.1 vs 2.3+/-0.3 mmol/l, p<0.05) levels, while we did not find the changes in HDL-Ch levels.
Conclusion: Our results have demonstrated that the weight loss in obese type 2 diabetic patients with CAD was strongly associated with important beneficial increases in the antioxidant enzyme activity. The beneficial effect is also found to be in association with the improvements in insulin sensitivity as well as in PI and in the serum lipoprotein excluding HDL-Ch levels.

PS 105

Macrovascular disease: genes and basic mechanisms

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The PAI-1 4G/5G polymorphism affects risk of complications in type 1 and type 2 diabetes with no interaction with the ACE I/D polymorphism
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Background and Aims: The plasminogen activator system is largely controlled by the renin-angiotensin system. Angiotensin II increases mRNA expression and activity of PAI-1. Both systems promote mechanisms extensively involved in complications such as proliferation of smooth muscle and mesangial cells and accumulation of extracellular matrix. ACE and PAI-1 activities are regulated by an I/D polymorphism in ACE gene and a 4/5 guanine tract polymorphism (4G/5G) in the promoter of PAI-1 gene, respectively. By multivariate logistic regression analysis, we aimed to investigate the independent or sinergistic effects of ACE ID and PAI-1 4G5G variants on the occurrence of nephropathy, retinopathy, hypertension and macrovascular disease (CVD) in 732 type 1 and 605 type 2 diabetic subjects.
Materials and Methods: The occurrence of disease of at least one of the three main vascular beds (coronary, peripheral, cerebrovascular) defined the presence of CVD. PCR and PCR-RFLP (BslI) were employed for ACE and PAI-1 genotyping, respectively. For a further analysis of the association with microangiopathy, subjects have been divided into three groups according to absence of any complication, the presence of at least one complication at an incipient stage (microalbuminuria and/or background retinopathy), or the presence of at least one at an advanced stage (overt nephropathy and/or proliferative retinopathy).
Results: In both type 1 and type 2 diabetes, PAI-1 4G/5G polymorphism was related to the presence of microvascular complications. In type 1 diabetes, the 4G4G genotype was more frequent in subjects with raised AER (34 vs 24%, p=0.01), proliferative retinopathy (35 vs 25%, p=0.03) and advanced or incipient renal and retinal microangiopathy (37% and 25% vs 18%; p<0.0001). In type 2 diabetes, at least in females, the 4G4G genotype was associated with overt nephropathy (p=0.02). In type 1 diabetes the relationship between the 4G4G genotype and any degree of renal involvement, proliferative retinopathy and combined advanced microvascular complications resulted independent of diabetes duration, blood pressure and HbA1c. In type 2 diabetes (mainly in females) the relationship of 4G5G polymorphism with nephropathy resulted independent of systolic blood pressure, HbA1c and BMI. In type 1 diabetes, but not in type 2, the frequency of the 4G4G genotype was higher in presence of CVD (47 vs 30%, p=0.003). The only significant association of ACE I/D was with hypertension and CVD in type 2 diabetes: indeed, the II genotype was more frequent in normotensives (19% vs 10%, p=0.01) and in subjects with no CVD (14% vs 7%, p=0.03). No relevant interaction of synergistic effects have been observed between the two polymorphisms in modulating the risk of micro- or macrovascular complications. Indeed, the distribution of the PAI-1 genotypes in relation to presence and severity of nephropathy or retinopathy or of any microvascular complication was similar for each ACE ID genotype. This was true both for type 1 as well type 2 diabetes.
Conclusion: These results suggest that the 4G5G PAI-1 polymorphism, but not the ID ACE variant, independently contributes to rise the risk of microvascular complications mainly in type 1 diabetes. Neither for micro- nor for macroangiopathy the biologically conceivable interaction between ACE ID and PAI 4G5G polymorphisms could be uncovered.

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Positive association of a genetic variant of the *APM1* with coronary artery disease
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Background and Aims: Adiponectin has been shown to possess insulin-sensitizing and anti-atherogenic effect. Interestingly, the genetic polymorphisms of adiponectin have been reported associated with insulin sensitivity, glucose homeostasis, obesity and type 2 diabetes mellitus. Although an

association of adiponectin gene polymorphism with coronary artery disease has been reported, there is no report in Chinese population. Our present study is aimed to confirm study the association between adiponectin gene polymorphisms with coronary artery disease in Chinese population.
Materials and methods: A group of 556 patients (441 men and 115 women) with angiographically diagnosed coronary artery disease (CAD) at National Taiwan University Hospital were recruited together with a group of 371 normals (220 men and 151 women) as control. A T94G polymorphism of exon 2 of the adiponectin gene was determined by polymerase chain reaction-restriction fragmented length polymorphism (PCR-RFLP).
Results: The genotypic frequencies for TT/TG/GG were 63.6% / 58.2% / 48.8% for CAD patients and were 36.4% / 41.8% / 51.2% for normal controls. (By Chi-square test, p=0.024) Logistic regression analysis showed that the CAD was significantly associated with adiponectin genotype (TT/TG vs GG, p=0.010) independent of age, sex, presence of hypertension, diabetes mellitus, smoking and total cholesterol level.
Conclusion: Our study confirmed that T94G polymorphism of the adiponectin gene conferred risk to CAD. The GG genotype of the adiponectin gene was protective against CAD in our population.

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ACE and PAI-1 insertion/deletion polymorphisms and age-related changes in pulse pressure in subjects with type 2 diabetes
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Background and Aims: Systolic blood pressure (SBP) and pulse pressure (PP), a marker of cyclic strain on the arterial wall, are independent predictors of CVD, mainly myocardial infarction. In diabetes, SBP and PP are also strictly related to kidney damage. For subjects over 50 years of age, SBP and PP increase exponentially with age, whereas DBP remains stable or tends to decrease. Recent studies suggest that the ACE I/D and the PAI-1 4G/5G gene polymorphisms modulate the age-related increase of PP in subjects with essential hypertension, potentially modulating the resulting vascular risk.
Materials and Methods: We aimed to investigate, in type 2 diabetes, the role of ACE I/D and PAI-1 4G/5G gene polymorphisms to the age-related changes in BP. The intergroup difference in the slope of the equation relating age and BP (SBP, DBP, PP) according to gender and, in a given gender, according to genotypes was checked by a test of heterogeneity of slopes. The study involves 605 type 2 diabetics (383 M, 222 F): age 61+/–8 years, DD 13+/–10 years, BMI 28.4+/–5.2 kg/m^2, HbA1c 8.7+/–1.8%. Hypertension was defined as SBP>140 mmHg or DBP>90 mmHg or use of antihypertensives. Urinary albumin was measured by an immunoturbidimetric method. PCR and PCR-RFLP (BslI) were employed for ACE and PAI-1 genotyping, respectively.
Results: ACE I/I 12%, I/D 49%, D/D 39%; PAI-1 5G/5G 21%, 4G/5G 48%, 4G/4G 31%. The Hardy-Weinberg equilibrium was mantained. No differences by gender in genotype distribution were observed. Hypertension and BP levels: hypertension was present in 80% of subjects (81% M, 77% F). Mean values of SBP, DBP and PP did not differ by gender and by genotypes. BP regressions on age by gender and genotypes: a negative marginally significant regression was observed between age and DBP (r=-0.10, p=0.03) with no differences by gender or genotypes. In the whole group, both SBP (r=0.28) and PP (r=0.40) correlated with age (p<0.0001), with no differences by gender. The slopes of the age-BP regressions (SBP and PP) did not differ in the three ACE I/D genotypes in females, while only marginally significant differences were observed in males (SBP: 0.94+/-0.49; 0.91+/-0.20 and 0.26+/–0.21 – PP: 0.89+/–0.36, 1.01+/–0.16 and 0.54+/–0.18 for II, ID and DD, respectively, p<0.05). The slopes of the age-BP regressions (SBP and PP) did not differ in the three PAI-1 4G/5G genotypes in males, while significant differences were observed in females (SBP: 1.31+/–0.32; 0.42+/–0.20 and 0.21+/–0.45 – PP: 1.11+/–0.23, 0.68+/–0.16 and 0.32+/–0.37 for 5G/5G, 4G/5G and 4G/4G, respectively, p<0.001). In females, PP was lower in 5G/5G than in 4G/5G and 4G/4G (60+/–17, 64+/–16 and 69+/–17 mmHg, respectively, p=0.05). PAI-1 gene and nephropathy: in females, not in males, the 4G4G genotype was associated with overt nephropathy (p=0.02). By logistic regression, the relationship of 4G/5G with nephropathy resulted independent of SBP (or PP), HbA1c and BMI.
Conclusion: In females with type 2 diabetes, the omozygosity for the 4G allele of the PAI-1 gene is associated with a lower slope in the regression between age and SBP or PP, higher PP levels independently of age and increased prevalence of overt nephropathy. PP may be a mechanical factor establishing a link between PAI-1 and renal damage, but the independent role of PP and the PAI-1 polymorphism suggest that this is only one of the possible pathogenetic mechanisms.

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The A-allele of the -866G>A variant in the promoter of UCP2 gene is associated with decreased prevalence of coronary heart disease in type 2 diabetic men
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Background and Aims: A common G/A single nucleotide polymorphism (SNP) at position -866 in the uncoupling protein 2 (UCP2) promoter region was shown to modulate mRNA expression in adipocytes and pancreatic beta-cells, with increased expression associated with the A-allele. Allelic associations with several phenotypes related to obesity, glucose homeostasis and dyslipidemia were observed. In rodents, UCP2 inhibits insulin secretion, lipogenesis in the adipose tissue, and the production of reactive oxygen species, notably in macrophages, and is associated with protection against atherosclerosis. In the present study, we investigated the association of the -866G>A SNP with coronary heart disease in a cohort of subjects with type 2 diabetes (T2DM).
Materials and Methods: We studied 243 unrelated French Caucasian T2DM men. Clinical and biological data were recorded. Coronary artery disease was assessed by coronary angiography. The SNP was examined by PCR-RFLP.
Results: CHD was present in 58% of patients. Subjects with CHD, as compared to subjects without CHD, were older (66 ± 10 vs 60 ± 10, p<0.0001, m±SD), had a longer duration of diabetes (14 ± 11 vs 11 ± 9, p<0.02), and were more often treated with insulin (38% vs 24%, p<0.02). They had a higher prevalence of increased LDL-cholesterol levels (67% vs 54%, p=0.004) and of arterial hypertension (86% vs 70%, p=0.001), and had higher levels of plasma creatinine and of urinary albumin excretion and lower creatinine clearance. There was also a higher prevalence of present or past cigarette smoking in subjects with CHD. The BMI, the prevalence of obesity, fasting plasma glucose and HbA1c levels were similar in both groups. The frequency of the A-allele was lower in subjects with CHD as compared with subjects without CHD, but this difference was not statistically significant (0.329 vs 0.379; p=0.29). There was a trend towards a decreased frequency of the AA genotype and increased frequency of the GG genotype in subjects with CHD (0.421 GG / 0.500 GA / 0.079 AA vs 0.398 GG / 0.447 GA / 0.155 AA, p=0.17). The age-adjusted prevalence of CHD according to genotype was 60% vs 61% vs 41% in G-allele homozygous, heterozygous, and A-allele homozygous subjects, respectively (p=0.04). In a logistic regressive model taking into account phenotype differences observed in subjects with or without CHD, age, increased LDL-cholesterol levels, decreased creatinine clearance and arterial hypertension were independently associated with CHD in this cohort. Homozygosis for the A-allele of the-866G>A variant was also independently and inversely associated with CHD (odds ratio of 0.37, 95% C.I. 0.14–0.84, p=0.019 as compared to other genotypes).
Conclusion: The A-allele of the-866G>A variant of UCP2 was associated with decreased prevalence of CHD assessed by coronary angiography in a cohort of men with T2DM. This association was independent from other common CHD risk factors.

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Circulating endothelial progenitor cells provide a marker of severity for peripheral arterial diseases in type 2 diabetic patients
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Background and Aims: Peripheral arterial disease (PAD) is a common and threatening complication of diabetes. Endothelial progenitor cells (EPCs) have been recognized as circulating precursors for adult neovasculogenesis and a role for EPCs in maintenance of vascular homeostasis has been recently shown. Given the poor collateral support and the altered endothelial function that are typical of diabetes, reduction in EPCs has been invoked in the pathogenesis of macrovascular complications of diabetes. Therefore, this study aimed to establish whether EPC decrease correlates with PAD severity in type 2 diabetic patients.
Materials and Methods: Circulating EPCs have been defined as CD34+KDR+ cells and counted by flow cytometry in sixty diabetic patients with and without PAD. PAD severity has been assessed as the carotid atherosclerotic burden and the clinical stage of leg atherosclerosis obliterans.
Results: Diabetic patients with PAD displayed a significant 50% reduction in EPCs in comparison with control diabetic patients. Moreover, EPC levels were negatively correlated with both the degree of carotid stenosis (*r*=-

0.66; $p<0.001$) and the stage of leg atherosclerosis (r=-0.61; $p<0.001$). Multiple logistic regression showed that EPC count was the only independent predictor of the presence of PAD in our patients. ROC curve analysis revealed that EPC count had a 75% sensitivity, 90% specificity and 85.2% accuracy in the diagnosis of PAD
Conclusion: This study demonstrates for the first time that EPC decrease is related to the severity peripheral vascular disease, strengthening the pathogenic role of EPC reduction in vascular disease of diabetes. Moreover, we propose that circulating EPCs may be considered a novel biologic marker of peripheral atherosclerosis in diabetes.

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Oleic acid reduces monocyte adhesion to activated glucose-treated human endothelial cells

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Background and Aims: The oleic acid-rich Mediterranean diet in contrast to the linoleic acid-rich Northern European diet may protect against cardiovascular disease by improving endothelial function. The aim of this study was to investigate the effects of oleic acid versus linoleic acid on adhesion molecule expression and subsequent monocyte binding in activated glucose-treated human endothelial cells.
Materials and Methods: HUVEC were grown in normal (5 mmol/l; N) or high (25 mmol/l; H) D-glucose for 6 days. Oleic or linoleic acid were added to growth medium (final conc 0.2 mmol/l) using fatty acid free BSA as a carrier (6:1) for the final 3 days of culture prior to TNF-α stimulation (10 ng/ml; 4–12 hr). L-glucose was used as a control. The cell surface expression of the adhesion molecules (E-selectin, ICAM-1 and VCAM-1) was quantified in fixed cells by ELISA. The number of fluorescently labelled monocytes bound to the treated endothelial cells was assessed by fluorescence microscopy. Real time PCR was used to quantify mRNA expression.
Results: TNF-α stimulation induced the cell surface expression of the adhesion molecules E-selectin ($p<0.05$), ICAM-1 ($p<0.05$) and VCAM-1 ($p<0.05$) in both glucose concentrations tested. Oleic acid decreased E-selectin ($p<0.05$), ICAM-1 ($p<0.05$) and VCAM-1 ($p<0.05$) cell surface expression whereas addition of linoleic acid resulted in a significant increase in E-selectin ($p<0.05$) expression. Oleic acid reduced the mRNA expression of ICAM-1 ($p<0.001$) and E-selectin ($p<0.05$) as well as the inflammatory mediators, MCP-1 ($p<0.05$) and COX-2 ($p<0.05$). All endothelial cells treated with linoleic acid produced significantly higher levels of the chemokine, IL-8 ($p<0.001$). The reduction in the cell surface expression of the adhesion molecules was accompanied by a decrease ($p<0.05$) in the number of monocytes bound to the activated endothelial cells.
Conclusion: The monounsaturated oleic acid may exert its anti-atherogenic effect by decreasing the expression of adhesion molecules and inflammatory mediators thereby reducing monocyte adhesion.
Support: Irish Heart Foundation

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Elevated circulating free fatty acids(FFAs) concentration stimulates NADPH oxidase P47-phox subunit gene transcription and translation in rat aorta

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Background and Aims: An elevated circulating FFAs concentration is known to enhance oxidative stress. But only a few previous studies have addressed the potential relationship between FFA and vascular oxidative stress in vovo. The present study investegated the relationship between FFA and oxidative stress in intralipid infused S-D rat and examined whether changes in NADPH oxidase P47-phox subunit's gene transcription and translation in vessel tissues are correlated with FFA infusing time course.
Materials and Methods: Three groups S-D rats underwent 6 hr, 12 hr, 18 hr, and 24 hr infusion respectively: Controls (C, n=12): saline alone; FFA (n=12): 20% intralipid (1.8uL/min) + heparin 0.72IU/min; GSH +FFA (n=12): GSH (1.2 mg/kg/min) and intralipid /heparin. Serum FFAs level and GSH/GSSG ratio was evaluated at baseline and after infusion. Thoratic aortas were harvested at the end of infusion and the NADPH oxidase P47-phox subunit gene transcription and translation in vascular tissure were examined by the methords of real-time PCR and western blot.
Results: Basal serum FFAs levels were similar in the various groups and they elevated 3 ~5 times in FFA group after infusion and maintained the higher level during infusion (327.5 ± 50.7umol/L *vs.* 1567.4 ± 273.6umol/L, P<0.05). The GSH/GSSG ratio decreased gradually and drop to the lowest point after 18 hr infusing (82.7 ± 33 *vs*.41.3?.89, P<0.05) in FFA group. There were no significant changes of serum FFAs level and GSH/GSSG ratio in C group. In GSH+FFA group, serum FFAs level (829.4 ± 72.9 mmol/L) and GSH/GSSG ratio (62.1 ± 2.3) were imtermediate between C and FFA group (P<0.05). NADPH oxidase P47-phox subunit gene transcription and translation in vessel tissue were enhanced which were correlated with serum FFAs level (r=0.75,P<0.05) and GSH/GSSH ratio (r=0.83, P<0.05) in FFAs group.
Conclusion: An elevated circulating FFAs concentration may enhance oxidative stress in vessel tissues which may be related with the increased risk of cardioavascular disease in patients with metabolic syndrome. The mechanism involved include, at least partly, the increased NADPH oxidase P47-phox subunit gene transcription and translation produced by FFAs.
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Complement C6 deficiency protects against diabetes-induced vascular damage in rats

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Background and Aims: Diabetes mellitus is associated with a marked increase in atherosclerosis. However, the mechanisms by which diabetes promotes vessel disease have not been fully delineated. Therefore, the aim of this study was to determine whether complement activation may contribute to vascular disease in short-term diabetes.
Materials and Methods: We used intravital videomicroscopy analysis of mesentery microvessels in four groups of 8 weeks old PVG rats (n = 8 for each group) weighing 250–270 grams which were studied for 2 weeks: 1. Non-diabetic PVG wild-type rats ($C6^{+/+}$), 2. Non-diabetic PVG C6-deficient rats ($C6^{-/-}$), 3. Streptozotocin-induced (50 mg/kg body weight) diabetic PVG wild-type rats ($DC6^{+/+}$) and 4. Diabetic PVG C6-deficient rats ($DC6^{-/-}$). Following intravascular cell fluorescent labelling with Acridin Orange, in vivo leukocyte trafficking at post-capillary venules was assessed in each animal, both in the absence or in the presence of an inflammatory stimuli (thrombin 0.125 U/ml).
Results: Post-hoc analysis of the recorded videomicroscopy images showed that diabetes in $C6^{+/+}$ rats was associated with an increased vascular adhesion of leukocytes to the inner vascular wall at basal conditions compared to the $C6^{-/-}$ animals. After exposure to thrombin, a significant increase of both transitory (58 ± 10 vs 38 ± 12 cells/200 μm; $p<0.01$) and stable (22 ± 8 vs 8 ± 3 cells/200 μm; $p<0.01$) leukocyte adhesion to vascular endothelium was observed in $DC6^{+/+}$ group compared to $DC6^{-/-}$ rats at 30 minutes. These effects were maintained over the entire course of the observation period (2 hours). In addition, leukocyte extravasation was significantly higher in $DC6^{+/+}$ rats compared to $DC6^{-/-}$ animals (12 ± 3 vs 6 ± 3 cells/400 μm^2; $p<0.05$) 2 hours after thrombin exposure. Diabetes *per se* was responsible for a significant increase of leukocyte trafficking at mesentery microvessels as assessed by the analysis of basal and thrombin-induced adhesion and extravasation in non diabetic control animals ($p<0.01$).
Conclusion: Our data demonstrate that the activation of terminal complement complex might be of relevance in the development of early inflammatory diabetic vascular disease, thus suggesting a potential new therapeutic target for the prevention of diabetes-induced atherosclerosis.

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Direct anti-atherogenic properties of PPAR alpha and PPAR gamma agonists

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Backgrounds and aims: PPARα agonists such as gemfibrozil are used to treat dyslipideamia whereas PPARγ agonists such as rosiglitazone treat insulin resistance. In addition, both PPARα and PPARγ agonists have been

postulated to confer cardiovascular benefits in diabetes, independent of their effects on glucose or lipid metabolism. We aimed to investigate the mechanisms underlying these anti-atherogenic effects in an *in vivo* model of diabetes-associated atherosclerosis with insulin deficiency.
Materials and methods: Control and streptozotocin-induced diabetic ApoE-/- mice were randomised to receive rosiglitazone (20 mg/kg/day) (C+R; D+R) or gemfibrozil (100 mg/kg/day) (C+G; D+G) by gavage or no treatment (C; D) for 20 weeks. Aortic plaque deposition was assessed by Sudan IV staining and subsequent *en face* quantitation. Superoxide production was measured using lucigenin-enhanced chemiluminescence. Gene and protein expression of markers of pro-atherosclerotic pathways were measured using real-time RT-PCR and immunohistochemistry, respectively.
Results: Diabetic mice had increased glycated haemoglobin (C,4.5 ± 0.2; D,17.2 ± 0.4% $p<0.0001$) and plasma cholesterol levels (C,12.1 ± 1.0; D,24.6 ± 0.9 mmol/L $p<0.0001$) and decreased plasma insulin levels (C,0.5 ± 0.1; D,0.2 ± 0.0 ng/mL $p<0.0001$) compared to control mice. Interestingly, there was no significant effect of R or G on glycated haemoglobin, insulin or cholesterol levels in the streptozotocin-diabetic mice. Diabetic ApoE-/- mice had a 3-fold increase in plaque area compared to control ApoE-/- mice which was significantly attenuated by both R and G (C,4.2 ± 1.0; D,12.5 ± 1.1; D+R,4.7 ± 0.9; D+G,1.2 ± 0.5% $p<0.0001$). This effect was most prominent in the aortic arch. NAD(P)H-dependent superoxide production was increased in diabetic mice (C,108 ± 7; D,165 ± 10 relative light units (RLU) $p<0.0001$) and this was significantly attenuated by both R (D+R,100 ± 10 RLU $p<0.0001$) and G (D+G,135 ± 14 $p<0.05$). Rosiglitazone treatment was also associated with significant reductions in the NAD(P)H subunit p47phox and angiotensin II type 1 receptor mRNA expression as well as macrophage infiltration in diabetic mice. In contrast, gemfibrozil treatment was associated with significant reductions in the mRNA expression of the NAD(P)H subunits p47phox and gp91phox as well as MMP-2 and MMP-9. In addition gemfibrozil reduced MCP-1 mRNA expression which may indicate that this agent is mediating some of its direct vascular effects via inhibition of NF-κB.
Conclusions: This study demonstrates that both rosiglitazone and gemfibrozil exert direct antiatherogenic actions, independent of changes in insulin, glucose and cholesterol. These agents have multiple actions involving effects on inflammation and oxidative stress. These results extend the utility of these agents potentially to a setting of atherosclerosis in the absence of dyslipidaemia or insulin resistance.

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Acarbose protects the vessel wall in experimental diabetes by reducing oxidative stress
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Background and Aims: Recent clinical studies provide evidence that treatment of patients with acarbose does not only diminish the risk for manifestation of diabetes, but also of cardiovascular complications associated with the metabolic syndrome. The pathophysiological mechanisms underlying vasoprotection by acarbose are however not yet fully understood.
Materials and Methods: 12 week-old obese Zucker rats (fa/fa, normoglycaemic, but hyperinsulinaemic) were kept on standard diet enriched in saccharose (10%); a part of the animals was treated with acarbose in chow (10 mg/kg body weight/day) for 7 days. As parameters of oxidative stress malondialdehyde (MDA) was determined in plasma (thiobarbituric acid method). To follow in vivo traces of oxidative stress in the vessel wall, the activity of mitochondrial aconitase (ACO) as a sensitive marker of oxidative stress in mitochondria was measured (optical test) in aorta and kidney ($n = 10$).
Results: As to be expected postprandial glucose levels were increased in obese Zucker rats as compared to lean littermates (244 vs 147, $p< 0.05$). In addition MDA was significantly elevated by 108% as compared to lean controls. Acarbose treatment reduced both the plasma levels of glucose (–17.6%) as well as of MDA (-38.5%) in obese ($p < 0.05$), but not in lean Zucker rats. ACO was reduced in aorta and kidney of obese Zucker rats, but was strongly increased by treatment of the obese rats with acarbose (from 14 ± 4 to 35 ± 6 mU/mg in aorta, and from 109 ± 7 to 142 ± 5 mU/mg protein in kidney, $p < 0.05$).
Conclusion: These data show that treatment with acarbose does not only diminish the hyperglycaemic load, but also the formation of reactive oxygen species (ROS). Our data suggest furthermore that hyperglycaemia causes an increase in the generation of ROS specifically by mitochondria leading to damage of the ROS sensitive aconitase. Maintenance of aconitase activity in vessel and kidney by treatment with acarbose can be understood as a consequence of reduced formation of mitochondrial ROS and a diminished uncoupling of mitochondrial electron transport caused by an overload with glucose. Reduction of ROS formation itself, but also of mitochondrial integrity are important mechanism to protect the vessel wall.

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Macrovascular disease: glucose and other risk factors

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The effect of high glucose on the proliferation and migration of vascular smooth muscle cells

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Background and Aims: Oxidative stress contributes to vascular diseases in patients with diabetes by promoting vascular smooth muscle cell (VSMC) proliferation, monocyte/macrophage infiltration, and vascular tone alteration. As the mechanism of development and progression of diabetic vascular complications is poorly understood, this study was aimed to assess the potential role of hyperglycemia-induced oxidative stress and to determine whether the oxidative stress is a major factor in hyperglycemia-induced migration and proliferation of VSMCs.
Materials and Methods: Rat aortic VSMCs were incubated for 48 hours in either normal glucose (NG, 5.5 mM) or high glucose (HG, 30 mM) condition. We measured the proliferation and migration of VSMCs and superoxide production.
Results: Migration and proliferation of VSMCs incubated under HG condition were markedly increased compared to NG condition. Treatment with diphenyleneiodonium (DPI, 10 μM) and superoxide dismutase (SOD, 500 U/mL) significantly suppressed HG-induced migration and proliferation of VSMCs. Superoxide production was significantly increased in HG condition and was markedly decreased after treatment with DPI and SOD.
Conclusion: These data suggest that HG-induced VSMC migration and proliferation are related to the production of superoxide anion derived from NAD(P)H oxidase.

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Glyco-oxidation and carotid atherosclerosis in type 2 diabetic patients

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Background and Aims: Progression of carotid atherosclerosis is accelerated in patients with diabetes. Potential mediators of vascular damage may include lipoprotein abnormalities (comprising a predominance of small dense LDL particles)), glycation and oxidation. The aim of this study was to assess the role of these factors in the progression of carotid atherosclerosis in type 2 diabetic patients.
Materials and Methods: 20 patients (11 men and 9 women, age 58 ± 13 years, duration of disease 4 ± 2 years, BMI 30.2 ± 5.9; values are expressed as mean±SD) without plaques, and 20 patients (13 men and 12 women, age 65 ± 9 years, duration of disease 7 ± 6 years, BMI 29.0 ± 4.6) with at least one carotid plaque, have been studied.
Carotid atherosclerosis was determined by high-resolution echocolordoppler. Internal right and left carotid arteries were studied in longitudinal and transversal projections in order to verify the presence of a plaque, understood as an endoluminal protrusion from the wall of more than 1.5 mm. As for glyco-oxidation end products, pentosidine was detected by Perkin Elmer HPLC system and carboxymethyllysine was evaluated by ELISA. LDL relative flotation, a measure of LDL particle buoyancy, was determined by density gradient ultracentrifugation. Nitrotyrosine, a marker of NO-dependent damage in vivo, was quantified by ELISA. Circulating oxidized LDL levels were measured by ELISA. IgG autoantibodies against oxidized LDL were detected by ELISA. To compare mean values Student's *t*-test was used. Analyses were conducted using the SAS software.
Results: The table shows that the presence of plaques was associated with small-dense LDL particles and higher concentrations of carboxymethyllysine and IgG autoantibodies against oxidized LDL.
Conclusion: In type 2 diabetic patients the presence of vascular disease seems to be associated with the presence of more oxidable LDL particles (small-dense LDL), of advanced glyco-oxidation end-product (carboxymethyllysine), and of an immunological response to oxidized lipoproteins (autoantibodies against oxidized LDL).

Table: Metabolic features of diabetics without and with plaques at carotid arteries

	Without plaques	With plaques	P
Glycosylated hemoglobin (%)	7.0 ± 1.2	7.0 ± 1.3	NS
Total cholesterol (mg/dl)	200 ± 37	212 ± 40	NS
HDL cholesterol (mg/dl)	53 ± 21	50 ± 14	NS
Triglycerides (mg/dl)	118 ± 52	144 ± 77	NS
LDL cholesterol(mg/dl)	124 ± 35	131 ± 36	NS
LDL relative flotation	0.36 ± 0.04	0.33 ± 0.04	0.008
Pentosidine (pmol/ml)	89 ± 44	99 ± 48	NS
Carboxymethyllysine (ng/ml)	1685 ± 173	1924 ± 360	0.01
Nitrotyrosine (nmol/L)	4.8 ± 0.9	4.7 ± 1.6	NS
Oxidized LDL (U/L)	42.6 ± 19.0	43.7 ± 12.4	NS
Autoantibodies against oxidized LDL (mU/ml)	193.2 ± 99.3	347.7 ± 293.0	0.04

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Serum glucose levels on admission in non diabetic patients with acute myocardial infraction represent an independent prognostic factor of the first year mortality

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Background and Aims: It has been shown that serum glucose levels on admission constitute an independent risk factor in non-diabetic patients undergoing an acute coronary syndrome. The purpose of this study was to examine the possible relation between admission's serum glucose levels and the mortality rate during the first year follow-up of non-diabetic patients.
Materials and Methods: A prospective study of 848 and 666 patients with STEMI and NSTEMI respectively, admitted to the hospital during the first 12 hours after the onset of pain was carried out. All patients had no previous history of diabetes mellitus and serum glucose levels on admission was <200 mg/dl. Patients with STEMI were separated into 3 groups according to the serum glucose levels, as follows: Group A: serum glucose levels <110, Group B: 110–126, Group C: 127–199. The same separation was performed in patients with NSTEMI.
Results: Overall mortality during the first year of follow up was 16.6% and 14.1% for STEMI and NSTEMI, respectively. Year mortality of the group A, B and C STEMI patients was: 11.2%, 16.2%, and 20.4%, respectively (p=0.002), while for the NSTEMI patients was: 8%, 12.6% and 19.1%, respectively (p=0.002). In multifactorial analysis serum glucose levels was found to be an independent mortality factor during the first year of follow up for both STEMI (p=0.007) and NSTEMI (p<0.001) patients.
Conclusion: The results of the present study show that serum glucose levels on admission represent an independent factor of increased mortality during the first year of follow up in non diabetic patients with acute myocardial infraction with or without ST elevation.

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Serum glucose levels on admission are related to the inflammation markers and myocardial necrosis in non diabetic patients with acute coronary syndromes

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Background and Aims: It has been found that serum glucose levels on admission represent an independent risk factor in non-diabetic (and in diabetic) patients that undergo an acute coronary syndrome. The underlying patho-physiologic mechanisms have not totally been explained. The purpose of this study was to estimate the possible relation between serum glucose levels, the levels of inflammatory markers and the myocardial necrosis in non-diabetic patients undergoing an acute coronary syndrome (STEMI or NSTEMI).
Materials and Methods: A prospective study including 848 and 666 patients with STEMI and NSTEMI, respectively that hospitalized during the first 12 hours after the onset of pain was carried out. All these patients had not any previous medical history of diabetes mellitus and initial serum glucose levels was <200 mg/dl. The patients were separated into three groups accord-

ing to the introduction serum glucose levels, as follows: Group A: serum glucose levels <110 mg/dl, Group B: serum glucose levels 110–126 mg/dl and Group C: serum glucose levels 127–199 mg/dl. At entrance the measured markers were: C-reactive protein (CRP), Interleukin-6 (IL-6), Fibrinogen (Fbg), White blood cells (WBC) and Troponin.
Results: It was found that there was a gradual increase of the values of CRP ($p<0.001$ and $p<0.001$ for STEMI and NSTEMI patients respectively), IL-6 ($p<0.001$ and $p<0.001$ for STEMI and NSTEMI patients respectively), Fbg ($p=0.02$ and $p=0.01$ for STEMI and NSTEMI patients respectively), WBC ($p<0.001$ and $p=0.001$ for STEMI and NSTEMI patients respectively) and Troponin ($p=0.007$ and $p<0.001$ for STEMI and NSTEMI patients respectively) from Group A to Group C accordingly and the findings were statistical significant for both the STEMI and NSTEMI patients.
Conclusion: The findings of the present study indicate indicate that the grade of the inflammatory response and of the myocardial necrosis is increased in proportion with the initial serum glucose levels in non-diabetic patients undergoing an acute coronary syndrome. The increased inflammatory response and myocardial necrosis are possible to be at least partially involved in worsening of the prognosis of these patients.

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Acute Myocardial Infarction and Diabetes (AMIDIAB) study: abnormalities of glucose homeostasis in patients with a positive history of acute myocardial infarction

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Background and Aims: Type 2 diabetes (DM2) is an independent classic risk factor for coronary heart disease (CHD). Others glycometabolic disturbances such as impaired glucose tolerance (IGT) are known to increase the risk for CHD mortality. The aim of this study was to evaluate the frequency of alterations in glucose metabolism in a Caucasian cohort of patients with history of AMI (not less than 3 months before their enrollment).
Materials and Methods: All the patients were from Central Italy and they were enrolled after exclusion of diabetes from their medical records. Enrolment of patients was initiated on February 2004 and continued until March 2005. Participants (N=126) were selected among patients visited during elective consultation at ambulatory of Internal Medicine and Cardiology of University of Tor Vergata. They had mean age 60,7 ± 9,3, mean BMI 27,6 ± 4,7, left ventricolar ejection fraction more than 50%. All the participants were treated with usual medical therapy for CHD, composed by aspirin, Ace-inhibitors or ATII blockers, statins, β-blockers and Ω-3. All the patients underwent to: 1) a coronarography and/or TC to determinate the number of vessels with stenosis >60% (1,2 or 3 vessel); 2) Oral glucose tolerance test (OGTT), 3) Insulin sensitivity and secretion indexes (HOMA IR, FIRI, QUICKI, Matsuda and HOMA beta); 4) HbA1C, C peptide and IGF-1; 5), lipid and pressure profile, 6) inflammation markers profile (VES, fibrinogen, high sensitivity PCR, TNF-α and CD40L).
Results: Upon OGTT, we observed that 26 (20,6%) patients were affected by DM, 48 (38,1%) were IGT, and only 52 (41,3%) were confirmed to be really normoglycemic (NGT). There were no gross significant differences in distribution of lipid and pressure profile, hepatic and kidney functionality, C peptide, VES, fibrinogen, hsPCR and among the 3 groups. Values of TNF-α CD40L and levels of HbA1C were significantly increased in DM2 patients compared to both IGT and NGT subjects ($p<0.05$ and $p<0,01$, respectively). Moreover, HOMA IR, FIRI and Matsuda, indexes of insulin resistance, were significantly higher in the group of DM ($p<0,05$), determining this group as the more insulin resistant compared to both IGT and NGT. Interestingly, also IGF-1 values were lower in DM group, but only than NGT ($p<0,05$) and not than IGT. Correlation analysis showed that the number of vessel disease was significantly correlated to postload glicemic value (Rho=0,27, $p=0.012$).
Conclusion: Our study suggests that glucose homeostasis disturbances are frequently undiagnosed in CHD patients. Since adequate metabolic control is necessary to reduce cardiovascular disease mortality, we propose that OGTT should be performed in every patient affected by CHD.

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Type 2 diabetes mellitus is not a coronary artery disease risk equivalent

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Background and Aims: An equally high risk to die from a myocardial infarction (MI) has been described for patients with type 2 diabetes mellitus (T2DM) without a history of MI as for non-diabetic patients with a prior MI by one study from Finland, but other studies have not confirmed T2DM as a coronary artery disease (CAD) risk equivalent. Cardiovascular risk among diabetic patients may vary considerably if coronary atherosclerosis is present or absent. We aimed at comparing cardiovascular risk between patients with T2DM who do not have CAD at angiography and patients with angiographically proven CAD who do not have T2DM.
Material and Methods: We therefore assigned 750 consecutive patients according to their coronary angiograms to one of four groups: DM-/CAD- (patients with neither T2DM nor CAD at angiography, n = 244), DM+/CAD- (patients with T2DM but without CAD, n = 50), T2DM-/CAD+ (patients without T2DM but with CAD, n = 342), and DM+/CAD+ (patients with both T2DM and CAD, n = 114). The incidence of fatal and non-fatal vascular events was recorded over 4 years.
Results: The incidence of vascular events averaged 20.1% in the 750 patients and was strongly affected by the angiographic state but not by the diabetic state: the proportion of patients with vascular events was similar in DM-/CAD- (8.9%) and DM+/CAD- (10.0%) patients ($p = 0.739$), but higher in DM-/CAD+ (23.8%, $p <0.001$) and DM+/CAD+ patients (40.2%, $p <0.001$) when compared to DM-/CAD-. Also, the incidence of vascular events was significantly higher in DM+/CAD+ than in DM+/CAD- ($p <0.001$) or DM-/CAD+ ($p <0.001$). Most importantly, patients with T2DM only (DM+/CAD–) had a significantly lower event rate than non-diabetic coronary patients (DM-/CAD+, $p = 0.034$).
Conclusion: Our 4-year prospective study provides strong evidence that T2DM is not a CAD equivalent: patients with T2DM and normal coronary angiography face only half the risk of non-diabetic CAD patients to develop an atherosclerotic event.

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Signature pattern of circulating chemokines can improve the identification of coronary artery disease

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Background and Aims: Epidemiological studies have shown inflammatory markers as predictors of outcome and response to therapy in patients with cardiovascular disease. However, current markers lack of sufficient accuracy in coronary artery disease (CAD) identification in the single subject. We hypothesize that specific patterns of circulating inflammatory markers produced inside the vessel wall can be used as an informative approach to estimate the extent of vascular inflammation and the presence of artery disease.
Materials and Methods: To address this issue, we designed a nested case-control study by randomly selecting 51 patients with recent myocardial infarction (MI) and 44 healthy control subjects from a larger population-based study on genetic determinants of atherosclerosis: the Heart Health and Heredity Study (HHHS). Extensive database has been constructed to include clinical variables and medication profile, as well as plasma concentration of glucose, insulin, and lipids.
Using plasma samples collected at the time of enrolment, we performed a parallel measurement of 9 human chemokines (Eotaxin, IP-10, MCP-1, -2, -3, and -4, IL-8, MIP1a, and RANTES) with a commercially available protein array.
Results: MI patients resulted more medicated than controls and were characterized by higher prevalence of dyslipidemia and hypertension, and a more insulin-resistant phenotype, with higher plasma insulin, slightly higher BMI, and larger waist circumference. Plasma chemokines concentration resulted significantly elevated in case subjects compared with controls, even after adjustment for clinical variables and pharmacological therapies.
To identify a profile of variables able to accurately discriminate between cases and controls, we performed several multivariate analyses that provided consistently similar results. Two-dimensional hierarchical clustering and principal component analysis showed CAD patients to cluster closely together, and highlighted co-expression of chemokines as the most evident factor driving the clustering process. Using Support Vector Machines algo-

rithm and bivariate statistics, we identified a consistent set of variables differentially represented between the two groups and we entered them in a stepwise Logistic Regression (LR) analysis. The results were validated with Linear Discriminant analysis and CaRT, and demonstrated that a model composed by top-expressed chemokines (MCP-4, MIP-1a, Eotaxin) and some basic clinical information was able to identify CAD patients with up to 94% sensitivity and 96% specificity when tested in a ROC curve, compared to about 86%-80% of the best single chemokine MCP-4, and a non-significant predictive ability of clinical variables or CRP.
Conclusion: Although larger studies are needed to confirm these findings, the measurement of circulating chemokines seems to predict the presence of atherosclerotic disease with a high degree of accuracy. The predictive power is sensibly higher when the informations coming from several markers measured at the same time is combined. Hence the parallel measure of several "candidate" proteins involved in vascular inflammatory response should be considered a sensitive approach to improve the currently available diagnostic markers of coronary artery disease.
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Twenty-four hour studies in patients with type 2 diabetes reveal postprandial elevations of P-selectin-positive microparticles
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Background and Aims: Postprandial plasma glucose- and triglyceride responses, which are elevated and prolonged in type 2 diabetes (DM2), are associated with increased cardiovascular disease (CVD) risk. P-selectin (CD62P) plays an essential role in the multicellular interactions that occur during thrombosis and inflammation and has been implicated in the pathogenesis of CVD. During apoptosis and activation, endothelial and blood cells release microparticles (MP). Elevated levels of MP circulate in patients at risk of CVD. Recently, we found postprandial elevations of MP numbers in healthy men, however the relation between postprandial metabolic derangements and MP levels in DM2 patients is unknown.
Materials and Methods: We monitored the 24 h profiles of various metabolites and of MP in 6 DM2 and 6 healthy males, following a standardised high-fat mixed breakfast (t=0 h), lunch (t=4 h) and diner (t=8 h), mimicking the real-life situation. Blood samples were collected before and at t=2, 4, 6, 8, 12, 16 and 24 h following breakfast. Numbers, cellular origin and composition of MP were determined by flowcytometry.
Results: In patients (mean age 55 (SD 6.2) yrs; HbA1c 6.7 (0.7) %) vs controls, plasma AUC glucose and triglyceride levels were increased (both $p<0.05$), with mean peaks in plasma glucose occurring at t=2, 6 and 12 h (11.6 (3.4), 10.2 (4.0), 11.8 (3.7) mmol/l, respectively), whereas triglycerides peaked at t=6 (2.86 (1.3) mmol/l) and remained elevated until t=12 h ($p<0.05$). Total numbers of platelet-derived MP were similar in both groups. In patients, however, CD62P-positive MP fractions increased 1.4- to 2.4-fold ($p<0.05$) at time points corresponding with postprandial hyperglycaemia, whereas CD63-positive MP remained unchanged, suggesting specific meal-induced platelet activation, i.e., secretion of α-granule but not lysosomal granule content.
Conclusion: Using a real-life approach we found an association between the postprandial dysmetabolic changes in DM2 and increased P-selectin-exposing MP levels, which may link the postprandial state to elevated CVD risk.
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Relationship between aortic distensibility with the classical and newer cardiovascular risk factors in subjects with type 2 diabetes
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Background and aims: Reduced aortic distensibility (AD) represents an early marker of atherosclerosis. AD is reduced with aging, smoking, and the presence of dyslipidaemia, hypertension as well as diabetes mellitus. No literature data exists concerning the association between AD and the newer cardiovascular risk factors in patients with type 2 diabetes mellitus (T2D). In this study we examined the relationship between AD and the classical as well as the newer cardiovascular risk factors in subjects with T2D.
Material and methods: A total of 100 subjects with T2D [mean±SD age: 59.3 ± 8.8 years; 49 males and 51 females; duration of diabetes 9.1 ± 7.4 years] were studied. AD was measured by high-resolution ultrasonography. Classical and newer risk factors [plasma fibrinogen, high sensitivity C-reactive protein (hs-CRP), homocysteine, microalbuminouria, HOMA-insulin resistance, uric acid, and white blood cells) for atherosclerosis were determined using standard methods.
Results: Multivariate linear regression analysis, after adjustment for a number of confounding factors such as sex, weight, blood pressure, smoking habits, plasma lipid, fibrinogen, hs-CRP and homocysteine levels, glomerular filtration rate as well as the number of white blood cell count, demonstrated a significant and independent association between AD and age ($b= -0.008$, $P<0.001$), height ($b= 0.6$, $P<0.001$), duration of diabetes ($b= -0.009$, $P<0.001$), plasma levels of uric acid ($b= -0.03$, $P=0{,}001$) and the albumin/creatinine ratio ($b= -0.0008$, $P=0.01$). These variables explained 58% of the variability of AD values.
Conclusion: In patients with type 2 diabetes, AD is partly related with modifiable risk factors for atherosclerosis. Interventions aiming at reducing microalbuminouria and plasma uric acid levels might result in improvement of the elastic properties of the aorta and the subsequent high risk for macrovascular complications in diabetes.

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Incremental predictive value of carotid ultrasonography in the assessment of coronary risk in a cohort of asymptomatic type 2 diabetic subjects
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Background and Aims: Consensus guidelines recommend cardiovascular risk assessment as the initial step of primary prevention. The aim of this study was to evaluate the incremental predictive value for coronary events conferred by carotid ultrasonography in addition to risk assessment by Framingham score and screening for silent myocardial ischemia in a cohort of type 2 diabetic patients.
Materials and Methods: 229 patients free of any cardiovascular complication with at least one additional cardiovascular risk factor were studied prospectively. At baseline, all patients had an exercise treadmill test, carotid intima-media thickness (IMT) measurement, and coronary risk assessment by Framingham score. Cardiovascular events were registered during a 5-year follow-up period.
Results: Age, carotid IMT, carotid plaques, number of risk factors, Framingham score and sub-optimal exercise ECG were associated with incident cardiovascular events ($p<0.05$). Carotid IMT was an independent predictor of cardiovascular events ($p=0.045$). The predictive value for coronary events was similar for carotid IMT and Framingham score as assessed by area under the receiver operating characteristic (ROC) curves. An improvement in risk prediction was conferred by addition of carotid IMT in a Cox model (global χ^2 increased from 14.1 to 18.1, $p=0.035$).
Conclusion: This prospective study confirms that carotid IMT is a marker of cardiovascular risk in this type 2 diabetic cohort, establishes that carotid IMT provides a similar predictive value for coronary events than Framingham score, and suggests that the combination of these two indexes significantly improves risk prediction for these patients.

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Cardiovascular risk and diabetes

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Cardiovascular risk in patients affected by diabetes type 1 and autoimmune thyroiditis

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Background and aims: Atherosclerosis is a complex disease and many mechanisms are involved in its pathogenesis. Besides the well known risk factors, such as hypertension, diabetes mellitus, smoking, dyslipidemia, recent studies have suggested that inflammation and immune response can be involved in the development and progression of atherosclerosis. In the present study we analyzed if in young patients with no cardiovascular risk factors the presence of autoimmune disease could determine endothelial alterations involved in the first step of atherogenesis. For this purpose we evaluated the intima-media thickness of the common carotid artery (IMT), as a marker of subclinical macrovascular damage, and high sensitive C reactive protein (hsPCR), as a marker of systemic inflammation.
Subjects and methods: We selected the following 4 groups of subjects:
Group 1: 18 patients affected by diabetes mellitus type 1 and autoimmune thyroiditis (mean age 31,7 ± 5,4 yrs; mean duration of diabetes 19,5 ± 8,2 yrs; mean duration of thyroid disease 7,9 ± 4,3 yrs)
Group 2: 18 patients affected by diabetes mellitus type 1 without thyroid autoimmunity (mean age 30,4 ± 4,8 yrs; mean duration of diabetes 17,5 ± 5,9 yrs)
Group 3: 18 patients affected by autoimmune thyroiditis without diabetes (mean age 32,5 ± 5,3 yrs; mean duration of thyroid disease 3,5 ± 2,9 yrs)
Group 4: 18 healthy subjects as a control group (mean age 29,7 ± 4,2 yrs)
All patients underwent clinical, biochemical and vascular study. An accurate anamnesis was obtained in order to investigate smoking habit, familiarity for cardiac diseases, medications or other concurrent diseases. We measured the following parameters: body mass index (BMI, Kg/m$^{2)}$, systolic and diastolic arterial blood pressure (PAS and PAD, mmHg), IMT (mm), serum cholesterol (mg/dl), HDL (mg/dl), triglycerides (mg/dl), glucose (mg/dl), fibrinogen (mg/dl), FT4 (ng/ml), TSH (μU/ml), autoantibodies anti TPO, autoantibodies anti Tg and hsPCR (mg/dl).
Results: In table 1 we summerized our study's results.

Results

	GROUP 1	GROUP 2	GROUP 3	GROUP 4	p
IMT	0.81 ± 0.12	0.67 ± 0.12	0.70 ± 0.09	0.57 ± 0.08	<0.001
TSH	2.9 ± 0.7	2.75 ± 1.3	2.27 ± 0.96	2.85 ± 0.97	n.s.
FT4	1.17 ± 0.15	1.2 ± 0.2	1.12 ± 0.96	1.1 ± 0.87	n.s.
FIBRINOGEN	282.5 ± 61.7	233.2 ± 40.3	289.1 ± 49.4	211.4 ± 20.2	=0.002
hsPCR	0.199 ± 0.18	0.136 ± 0.14	0.046 ± 0.02	0.053 ± 0.01	=0.02
TOT. CHOLESTEROL	190.6 ± 27.8	188.3 ± 32.9	191.8 ± 25.6	187.8 ± 20.3	n.s.
HDL	64.3 ± 14.6	56.6 ± 9.7	65.9 ± 11.9	63.3 ± 12.4	n.s.
TRYGLICERIDS	73.5 ± 32.9	81.3 ± 26.4	79.8 ± 41.1	78.9 ± 31.2	n.s.
LDL	111.6 ± 22.9	115.3 ± 38.1	109.2 ± 22	108.3 ± 23.4	n.s.
PAS	120.8 ± 11.5	120.2 ± 8	113.8 ± 9.6	289.1 ± 49.4	n.s.
PAD	77.5 ± 9.4	78.7 ± 6.4	70.1 ± 8.6	77.4 ± 8.5	n.s.

Conclusions: This is the first study that showed an increase in IMT, marker of early macrovascular damage, in subjects affected by thyroiditis, without cardiovascular risk factors. This study also showed that the concomitant presence of thyroiditis in patients affected by diabetes type1 seems to aggravate the macrovascular alteration. In conclusion these data suggest that the presence of autoimmune disease could represent an adjunctive cardiovascular risk factor, underlining the important role of autoimmunity in the development and progression of atherosclerosis.

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Type 2 diabetic patients needing revascularization also/only at the infra-popliteal site present a poorer cardiovascular outcome than patients needing revascularization only at supra-popliteal sites

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Background and Aims: Diabetic patients with peripheral arterial obstructive disease (PAOD) present a severe cardiovascular risk profile. Aim of this study was to evaluate, in a consecutive series of diabetic patients with PAOD submitted to revascularization procedures, the outcomes at follow-up in terms of new cardiovascular events (angina, myocardial infarction, transient cerebral ischaemic attaks, stroke and revascularization at any site) and mortality in relation to the revascularization site. In particular, we aimed at investigating whether the need of revascularization under the knee -likely indicating the involvement of more distal and smaller vessels- further increases the cardiovascular risk.
Materials and Methods: We evaluated all the type 2 diabetic patients submitted to revascularization procedures at our Diabetes Unit in the years 1999–2004: n=77, 69.2 ± 8.9 yrs (m±SD), M/F 49/28, known diabetes duration 18.3 ± 11.5 yrs, smoking history 69%, arterial hypertension 86%, on diet treatment alone 11.7%, on oral agents 23.4%, on oral agents+ insulin 7.8%, on insulin 57.1%. Patients were submitted to revascularization because of PAOD at stages IIb-IV of the Lériche-Fontaine classification; ulcers were present in 55/77 patients. Percutaneous angioplasty was performed in 67/77 subjects (87%), surgical by-pass in 10/77 (13%); 43/77 revascularization procedures (55.9%) were performed only at a supra-popliteal site (Group A), 34/77 (44.1%) at a infra-popliteal or both supra- and infra-popliteal sites (Group B). Transcutaneous pressure of oxygen ($TcPO_2$) at the affected foot was higher in Group A than in Group B (30.6 ± 13.8 vs 17.1 ± 7.6 mm Hg, p=0.01). All patients were treated by the same team of diabetologists, vascular radiologists and vascular surgeons, and followed-up for 38.9 ± 23.7 months (range 1-79).
Results: 46/77 patients (59.7%) presented pain disappearance and ulcer healing without amputations after revascularization; 30/77 (39%) needed minor amputations; 1/77 (1.3%) a major (above the knee) amputation; 24/55 patients with ulcers (44%) healed without any amputation. During the follow-up, 32/77 patients (41.5%) presented a first cardiovascular event: 15/43 in Group A (35%) and 17/34 in Group B (50%), the mean event-free time being 43 ± 5 months in Group A and 35 ± 6 months in Group B (p=0.041). 14/77 (M8/F6) patients (18%) died, 10 because of a cardiovascular event, 1 because of respiratory failure, 1 because of kidney failure, and 2 because of sepsis: in particular, 5/43 subjects of Group A (11.6%) and 9/34 subjects of Group B (26.5%) died, mean survival time being 69 ± 4 months in Group A and 53 ± 6 months in Group B (p=0.03).
Conclusion: Among diabetic patients submitted to revascularization procedures for severe PAOD, the need of revascularization also/only at the infra-popliteal site identifies a subset of subjects at a greater risk of cardiovascular events than those needing revascularization only at the supra-popliteal site, likely because the presence of critical arterial stenosis under the knee indicates a more extensive and severe vascular damage also in the other vascular districts.

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Endothelial function, 24-hours blood pressure and cardiovascular autonomic function in type 1 diabetes mellitus

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Background and aims: Studies assessing endothelial function in patients with type 1 diabetes (DM1) have provided conflicting results depending on duration of disease, metabolic control, presence of microvascular complications and autonomic neuropathy. DM1 patients frequently have an altered circadian blood pressure (BP) profile with a blunted fall in night-time BP and an abnormal cardiovascular autonomic regulation, both of which may influence endothelial function.
This study examines the relation between endothelial function, 24-h blood pressure pattern, and heart rate variability (HRV) in DM1 patients free from micro- and macrovascular complications.
Material and methods: 7 healthy control subjects (C) (age 27 ± 2y, BMI 21 ± 3 kg/m^2) and 16 DM1 patients (age 35 ± 10 y, BMI 24 ± 2 kg/m^2, dia-

betes duration 15 ± 8 years, HbA1c 8.1 ± 1%) were studied. All subjects underwent the following measurements: 1) endothelium-dependent (flow-mediated) and endothelium-independent (nitroglycerin-induced) vasodilation of the brachial artery by ultrasonography; 2) 24-h BP recording; 3) HRV by time domain method.
Results: Flow-mediated dilation (FMD) was slightly but not significantly lower in DM1 patients (9 ± 5%) than in C (13 ± 2%; p=0.076), while endothelium- independent vasodilation (NMD) was similar in the two groups (12.7% and 12.4% in N and D, respectively; p= ns). Mean 24-h arterial BP was similar in N (113/76 mmHg) and D (121/77 mmHg). However, 7 DM1 patients (44%) showed an abnormal blood pressure fall during night-time (<10% than day-time levels) and were classified as non dippers, whereas all C were classified as dippers. Time domain HRV indexes were significantly different in the two groups (C vs D: SDANN 147 vs 106, SDNN 155 vs 119, RMSSD 38 vs 28, all p<0.01; pNN50 12 vs 8, HRVindex 36 vs 35, p=ns). In DM1 patients, no correlation was found between FMD and HRV indexes and PA parameters (24 hours, daytime, night-time and night-time/day-time average value). A positive correlation was found between NMD and night-time/day-time diastolic PA.
Conclusions: In DM1 patients clinically free from micro and macro-vascular complications, HRV is significantly reduced compared with C. A consistent proportion (44%) of the patients studied have a reduced nocturnal BP fall without significant impairment of endothelial vasodilation. The data indicate that BP regulation and CV autonomic function do not affect endothelial function in young DM1 patients.

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Serum mannose-binding lectin as predictor of mortality in type 2 diabetes

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Background and Aims: Inflammation and complement activation initiated by mannose-binding lectin (MBL) may be implicated in the pathogenesis of cardiovascular disease. We evaluated the association between serum MBL and mortality in a prospective observational study of 328 patients with type 2 diabetes.
Materials and Methods: Serum MBL and C-reactive protein (CRP) were measured using highly sensitive immunoassays at baseline in 328 patients with type 2 diabetes who attended the Steno Diabetes Centre for control between January and December 1987. The vital status of all patients was traced through linkage to the Danish Civil Registration System at the beginning of 1998, and the cause of death was obtained from the death certificates that were reviewed independently by two observers.
Results: During a median (interquartile range, IQR) follow-up time of 10.6 (8.0–10.8) years 113 (34%) patients died, predominantly from cardiovascular disease. Median (IQR) serum MBL concentrations were significantly higher at baseline in patients who later died than among survivors; this was seen both in the entire cohort: 1056 (260–2124) µg/l vs. 579 (153–1577) µg/L, p=0.023, and when patients with normoalbuminuria at baseline were analyzed separately: 1502 (291–2425) µg/L vs. 547 (131–1526) µg/L, p=0.008. From ROC curve analysis the best discriminative cut-off value for MBL as a predictor of mortality was identified as 1000 µg/L (area under the ROC curve 0.59, P=0.012). When dividing patients according to this cut-off the risk of dying during follow-up was 42.3% in patients with serum MBL above 1000 µg/L, as compared to 28.6% in patients with MBL levels below 1000 µg/L; hazard ratio 1.59 (1.10 to 2.30), log rank test: p=0.014. Among patients with normoalbuminuria at baseline, those with a baseline MBL above 1000 µg/L had a risk of dying during follow-up of 31.6% compared to 13.0% in patients with MBL levels below 1000 µg/L, hazard ratio 2.55 (1.34 to 4.87), log rank test: p=0.003. In a multivariate Cox proportional-hazards model with adjustment for the confounding effects of gender, age, known diabetes duration, systolic and diastolic blood pressure, BMI, serum total cholesterol, HbA_{1c}, urinary albumin excretion and smoking, a serum MBL level above 1000 µg/L remained an independent risk factor for dying both in the entire cohort [relative risk 1.49 (95% CI 1.01–2.21), p=0.04] and among patients with normoalbuminuria [relative risk 2.53 (1.27–5.07), p=0.009]. MBL added significantly to the predictive power of CRP, and patients with high MBL (>1000 µg/L) and high CRP levels (above the median, 3.6 mg/L) had a risk of dying of 45.6% compared to 18.3% among patients with both low MBL and low CRP levels, log rank test: p=0.001. Among patients with normoalbuminuria at baseline this difference was even more pronounced with a mortality of 39.4% in the high MBL, high CRP group vs. 7.7% in the low MBL, low CRP group, log rank test: p=0.003.
Conclusion: Measurement of serum MBL in patients with type 2 diabetes provides strong prognostic information on mortality that is independent of previously known risk factors.

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A novel indicator of widespread endothelial damage and ischemia in diabetic patients: ischemia modified albumin

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Background and Aims: Microalbuminuria is the first clinical sign of diabetic nephropathy. However, microalbuminuria is also a strong predictor of cardiovascular risk in both diabetic and non-diabetic individuals. Therefore microalbuminuria could be accepted as an indicator of widespread endothelial damage. Ischemia modified albumin (IMA) is a novel marker of tissue ischemia. It has shown to be increased in acute ischemic events. In this study we planned to assess the correlation of IMA and microalbuminuria in addition to other macrovascular risk factors in diabetic patients without overt macrovascular disease.
Materials and Methods: Fifty-two diabetic patients without a history of macrovascular disease or overt nephropathy were enrolled into the study. Age matched 30 healthy individuals were also included in the study as a control group. Both groups were evaluated with anthropometric measurements, metabolic parameters, C-reactive protein (CRP), and IMA. In addition to those plasma homocystein and microalbuminuria levels were studied in diabetic subjects. Presence of neuropathy and retinopathy were evaluated by specific tests.
Results: Age, body mass index, and serum lipid levels were not different between the two groups. Plasma IMA (0,3446 ± 0,03947 and 0,2652 ± 0,04504 AbsU, p<0,0001) and CRP levels (0,47 ± 0,41 and 0,32 ± 0,18 mg/dl; p=0,041) were significantly higher in the diabetic group compared with healthy controls. IMA levels were significantly correlated with CRP (r=0,57;p=0,012) and plasma homocystein (r=0,46;p=0,004) levels in diabetic subjects. In the diabetic patients group presence of microalbuminuria was associated with a higher plasma IMA level (0,3598 ± 0,03600 and 0,3261 ± 0,0366 AbsU, p=0,011, patients with or without microalbuminuria, respectively). We did not find any specific association of plasma IMA levels with retinopathy or neuropathy.
Conclusion: Diabetic patients without an overt cardiovascular disease still have a higher serum IMA level in comparison with healthy controls. The correlation of high plasma IMA levels with high CRP and homocystein levels in diabetic subjects indicates the presence of a chronic ischemic process. Therefore diabetes should be regarded as a cardiovascular risk equivalent as indicated in the recent cardiovascular disease guidelines.

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Do process and intermediate outcome measures predict the incidence of long-term cardiovascular events in type 2 diabetes? Results of the QuED Study

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Background and Aims: Several public and private health care organizations have promoted initiatives to measure and improve the quality of care for patients with diabetes. It is not clear to what extent process and intermediate outcomes measures adopted predict long-term effects on patients' health. In the framework of the QuED Study we evaluated whether widely accepted indicators of diabetes care were able to predict the development of cardiovascular (CV) events over 5 years.
Materials and Methods: We evaluated whether HbA1c, blood pressure, LDL cholesterol, and microalbuminuria had been measured in at least one occasion in the last 12 months (process measures) and whether specific therapeutic goals had been attained (intermediate outcomes). Incident CV events considered included: angina, myocardial infarction, stroke, TIA, coronary revascularization procedures, lower limb complications, aortic-femoral revascularization procedures, CV mortality. We used multilevel Poisson regression models to investigate whether the indicators were independent predictors of CV events incidence. Analyses were adjusted for patient case-mix and physician-level clustering.

Results: Overall, 3031 patients with type 2 diabetes were evaluated, of whom 492 (14.0%) developed a CV event during the follow-up. Results of multivariate multilevel analyses are reported in the table (for each indicator the reference category is represented by the attainment of the respective therapeutic goal).
Conclusion: The study documented for the first time that both process and intermediate outcome measures predict long-term CV complications. In particular, not measuring HbA1c and lipid profile was associated with a substantial increase in the risk of CV events. Not reaching specific targets in terms of metabolic, blood pressure, and lipid control was also associated with an increased risk of overall or specific CV events. Finally, the lack of ACE-inhibitors treatment in the presence of microalbuminuria also represented an important predictor of CV events. The predictive role of the indicators selected was not homogenous and varied according to the type of event considered. In the evaluation of quality of care, it is necessary to attribute different weights to the selected indicators according to the outcomes considered.

Process and intermediate outcome measures	Long-term events (Relative Risk and CI 95%) Total events	Major events	CV events	Cerebro-vascular events	LLCs	CV Mortality
HbA1c not measured	1.38 (1.02–1.9)	1.50 (1.02–2.2)	1.95 (1.3–2.9)	1.09 (0.6–)1.9	1.37 (0.8–2.4)	1.06 (0.5–2.2)
HbA1c ≥8%	1.45 (1.2–1.8)	1.46 (1.1–2.0)	1.72 (1.2–2.4)	0.74 (0.5–1.2)	1.52 (1.0–2.3)	1.28 (0.8–2.1)
Blood pressure not measured	0.28 (0.1–0.9)	0.33 (0.08–1.4)	0.22 (0.03–1.6)	0.78 (0.2–3.4)	0.34 (0.05–2.5)	0.99 (0.2–4.4)
Blood pressure ≥160/100 mmHg	1.16 (0.9–1.5)	1.40 (0.98–2.0)	1.42 (0.95–2.1)	1.80 (1.04–3.1)	1.04 (0.6–1.8)	0.98 (0.5–1.8)
Lipid profile not measured	1.33 (1.02–1.7)	1.42 (1.01–2.0)	1.34 (0.9–2.0)	1.38 (0.8–2.3)	1.53 (0.95–2.5)	1.22 (0.7–2.1)
LDL Cholesterol ≥130 mg/dl	1.32 (1.03–1.7)	1.45 (1.06–2.0)	1.53 (1.1–2.2)	1.57 (0.96–2.6)	1.08 (0.7–1.7)	1.00 (0.6–1.7)
Microalbuminuria not measured	1.14 (0.9–1.4)	1.13 (0.9–1.5)	1.14 (0.8–1.6)	1.15 (0.8–1.8)	1.12 (0.7–1.7)	1.08 (0.7–1.8)
Microalbuminuria present, no ACE-inhibitors	1.54 (1.08–2.2)	1.55 (1.0–2.4)	1.31 (0.8–2.2)	1.50 (0.7–3.1)	2.03 (1.1–3.7)	1.16 (0.5–2.6)

Major events: AMI + stroke + CV mortality; LLCs= lower limb complications.

Support: Pfizer Italiana S.p.A.

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Increased plasma hyaluronan as novel predictor for atherosclerotic vulnerability in type 1 diabetes mellitus

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Background and Aims: Atherosclerotic macrovascular disease contributes significantly to overall mortality in type 1 diabetes mellitus (DM1). These patients show increased vulnerability for atherogenic insult, possibly related to increased vascular permeability. Recently we found that plasma hyaluronan (the most abundant proteoglycan in the endothelial glycocalyx) is inversely correlated with systemic glycocalyx volume. To assess whether increased plasma hyaluronan levels, as marker for perturbed endothelial glycocalyx, may contribute to increased atherogenic vulnerability and chronical vascular inflammation in DM1, we studied the correlation between intima media thickness (IMT), plasma hyaluronan levels and hsCRP in DM1 versus healthy controls.
Materials and Methods: We recruited non smoking DM1 patients free of concomitant illnesses. Subjects with retinopathy, neuropathy, microalbuminuria, hypertension or hypercholesterolaemia were excluded. All patients were on multiple daily injections of insulin. Plasma hyaluronan and hsCRP levels were determined by ELISA. B-Mode ultrasound IMT of several sections of carotid wall of both arteries was performed. Student's t-test (normal distribution) or Mann-Whitney test (non parametric) were used for comparison between DM1 and controls. To evaluate the relation between Intima Media Thickness and other parameters, simple or multiple regression analyses were performed. Predictive variables for the occurence of carotid plaques were determined by logistic regression analysis. $P < 0.05$ was considered significant.
Results: We included 99 DM1 patients (age range 10–72 years) and 123 unrelated controls matched for age and gender. Mean duration of DM1 was 16.4 ± 11.8 years. Age, gender distribution, BMI, bloodpressure and transaminases were similar. HbA1c (8.3 ± 1.6 vs. 4.9 ± 0.4%, $P<0.001$), hsCRP (2.6 ± 0.4 vs. 1.6 ± 0.2 mg/l, $P<0.05$), hyaluronan (78.0 ± 43.6 vs. 62.0 ± 19.4 ng/ml, $P<0.05$) were significantly increased in the diabetes group. Mean B-mode IMT of all carotid arterial wall segments was significantly increased in DM1 versus controls (0.61 ± 0.02 vs. 0.52 ± 0.01 mm, $P<0.001$). Using simple and multivariate regression analyses mean IMT correlated significantly with plasma hyaluronan, hsCRP and age respectively ($P<0.05$).
Conclusion: DM-1 patients show structural changes of the arterial wall and increased plasma hyaluronan levels and both parameters are signifantly correlated. Moreover, these patients have signs of an increased inflammatory state, which might offer an explanation for increased plasma hyaluronan. We therefore conclude that plasma hyaluronan can be regarded as a novel marker for cardiovascular risk and that loss of systemic glycocalyx volume in DM1 patients may explain their increased atherogenesis.

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Effect of severe weight loss on non-traditional cardiovascular risk factors in morbidly obese subjects

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Background and Aims: Obesity is an independent risk factor for cardiovascular disease (CVD) and is frequently associated with several features of the metabolic syndrome. In particulary, obese subjects carry a proinflammatory state that may promote early atherosclerosis and CVD. It is suggested that weight loss might influence diagnosis in this population due to reduction of well-known risk factors, e. g hypertension or dyslipidemia. The aim of this study was to examine the effect of weight loss following bariatric surgery on non-traditional cardiovascular risk factors.
Materials and Methods: 32 morbidly obese patients (29f/3 m, 42 ± 9years, BMI:46.1 ± 5.85 kg/m^2) were treated with gastric banding (GB) and various non-traditional risk markers were measured before, 6 and 12 months after surgery.
Results: A mean weight loss of 32.1 kg resulted in a significant reduction of traditional risk factors (waist, systolic and diastolic blood pressure; all $p<0.0001$, fasting blood glucose; $p=0.02$, triglycerides;$p=0.002$). In addition, adhesion molecules VCAM-1 ($p=0.009$) and E-Selectin ($p=0.002$) were significantly lowered 12 months after GB, while ICAM-1 decreased only significantly after 6 months. CD40ligand, Lp-PLA$_2$ and MMP-2 showed no changes, whereas MMP-9 levels were significantly lower ($p=0.05$) 1 year after GB.
Conclusion: Except for adhesion molecules and MMP-9, non-traditional cardiovascular risk markers did not change after pronounced weight loss following GB. These results support previous findings, that weight loss might not completely normalize the proinflammatory state in morbidly obese subjects. On the other side, non-traditional risk markers, representing instable plaque formation might be less sensitive in an early stage of endothelial injury as represented by this population.

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Anti-atherosclerotic and renoprotective effects of rosuvastatin in a model of diabetes- accelerated atherosclerosis

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Background and Aims: Macrovascular disease accounts for the majority of deaths in the diabetic population. Cardiovascular risk is even higher in the context of underlying renal disease and microalbuminuria. It has been hypothesized that statins exert direct vascular effects independent of their lipid lowering properties. The issue if statins prevent the onset or progression of diabetic nephropathy is still controversial. In this study we have investigated the anti-atherosclerotic and renal effects of the novel HMG CoA reductase inhibitor, rosuvastatin, on diabetes accelerated atherosclero-

sis and renal disease. Effects are compared to those of the AT1 receptor blocker, candesartan. We use the diabetic apoE KO mouse, a model combining hyperlipidemia and diabetes that has previously been reported to be resistant to HMG CoA reductase treatment.
Materials and Methods: Diabetes was induced in apoE KO mice by streptozotocin injections (5 days, 55 mg/kg intraperitoneally). Non-diabetic and diabetic apoE KO mouse were left untreated or were treated with candesartan (2.5 mg/kg BW) or rosuvastatin for 20 weeks (5 mg/kg BW) per gavage. At the end of 20 weeks of diabetes animals were weighed, blood pressure was measured and blood samples were collected for lipids, glucose and HbA1c as well as aortas collected. Plaque area in the aorta was analysed by the en face approach after Sudan IV staining by computerized image analysis. Albuminuria was measured by RIA as described previously. * p< 0.05, ** p< 0.01 vs apoE KO+ diabetes
Results:

RESULTS

	HBA1C (%)	Cholesterol Âµmol/L	Plaque area (%)	alpha-SMA (%)	CTGF (%)	Albuminuria Âµg/24 hours
apoE KO + diabetes	11.6Â± 0.8	30.6Â± 2.3	12.5Â± 1.1	3.9Â± 0.5	0.94Â± 0.27	50.6Â± 3.5
Diabetes + candesartan	12.1Â± 0.7	27.1Â± 1.9	2.8Â± 1.2**	5.4Â± 2.6	0.23Â± 0.1**	28.2Â± 3**
Diabetes + rosuvastatin	12.3Â± 0.5	24.1Â± 1.6*	4.5Â± 1.7**	1.5Â± 0.6**	0.24Â± 0.08**	41Â± 3.4*

Conclusion: The HMG CoA reductase inhibitor rosuvastatin had anti-atherosclerotic effects similar to the AT1 receptor blocker candesartan in a model of diabetes accelerated atherosclerosis, the diabetic apoE KO mouse. The anti-atherosclerotic effects of rosuvastatin were associated with reductions in the number of alpha-smooth muscle actin positive cells, inflammatory parameters and the expression of the pro-fibrotic growth factor connective tissue growth factor (CTGF). Furthermore, rosuvastatin also reduced albuminuria and other parameters of renal injury in this model.

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Thrombocyte function and free radical production of type 1 and type 2 diabetic patients with peripheral arterial diseases; the effect of insulin in vitro
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Background and aims: Insulin influence not only carbohydrate and lipid metabolism, but also regulates thrombocyta function and vascular tone. It activates constitutive NO synthase and due to the elevation of intracellular cGMP and cAMP levels it causes antiaggregating and vasodilator effects. Insulin is able to influence free radical production in a positive and negative way. The aim of the present study was to investigate the thrombocyta function and free radical production of type 1 and type 2 diabetic (1DB and 2DB) patients with peripheral arterial diseases. The other aim was to study how in vitro insulin can modify these parameters.
Patients and methods: 1DB and 2DB patients with peripheral arterial diseases and with other cardiovascular complications were involved in the studies. Thrombocyta function was investigated in whole blood and platelet rich plasma. Measurements were based on turbidimetric methods, and on impendance changes. ADP in 5 and 10 µM, Collagen in 2 µg/ml and Adrenaline in 10 µM were used as inductors. Superoxide production was induced by PMA and was measured by lumino-aggregometer. Insulin has a well established inhibitory effect on collagen induced aggregation, the effect of 40, 80 and 160 µU/ml quick-acting insulin was investigated on collagen induced aggregation. Insulin action on superoxide production was also measured. The members of antioxidant defence system (activity of SOD, concentration of GSH and MDA) were measured by conventional photometric methods.
Results: ADP and collagen produced less aggregation in whole blood of 2DB diabetic group. Exogenous insulin decreased collagen induced aggregation in both groups on whole blood. PMA induced maximal superoxide production corrected to white blood cell count was lower in 2DB group. SOD activity were lower in type two diabetic patients.
Conclusions: In the mirror of the measured parameters insulin deficient and insulin resistant cases were distinguishable in spite of the accompanied diseases. Antiaggregating and superoxide production lowering effects of insulin were demonstrable on patients with serious complications.

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Cardiovascular disease: metabolic syndrome and other risk factors

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Metabolic syndrome accompanied by hypercholesterolemia accelerates proinflammatory state and impairment of fibrinolysis in patients with type 2 diabetes: synergistic effects of PAI-1 and TAFI
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Aims: To investigate whether plasma concentrations of thrombin-activatable fibrinolysis inhibitor (TAFI), a new inhibitor of fibrinolysis, were associated with components of insulin resistance, including high sensitivity (hs)-CRP and PAI-1, or LDL cholesterol in patients with type 2 diabetes
Materials and Methods: We studied 136 consecutive patients with type 2 diabetes. Diagnosis of metabolic syndrome (MS) was made based on NCEP-ATP III criteria. Hypercholesterolemia (HC) was defined as serum low-density lipoprotein (LDL) cholesterol of exceeding 140 mg/dl (3.6 mmol/liter) or alternatively as treatment with a statin. On the basis of the definition of MS and HC, the diabetic patients were divided into four groups: diabetic patients with no MS /no HC (n=38); those with MS/no HC (n=39); those with no MS/HC (n=26) and those with MS/HC (n=33).
Results: In all patients with type 2 diabetes, plasma PAI-1 was associated strongly with components of MS such as BMI, triglyceride, ALT, HOMA-IR, and hs-CRP, whereas plasma TAFI correlated positively and independently with only LDL cholesterol. Plasma concentrations of plasmin-α2-antiplasmin complex (PAP), a measure of fibrinlytic activity in blood, showed a significant negative correlation with plasma PAI-1, but not TAFI. Diabetic patients with both MS and HC had the highest serum hs-CRP concentration and the lowest plasma PAP concentration among the four groups.
Conclusions: LDL cholesterol is a main determinant of plasma TAFI in patients with type 2 diabetes. The coexistence of metabolic syndrome and hypercholesterolemia accelerates synergistically inflammation and impaired fibrinolysis in these patients via elevated concentrations of both TAFI and PAI-1, inhibitors of fibrinolysis.

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Resistin and diabetic macroangiopathy among Japanese type 2 diabetic patients with or without metabolic syndrome
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Background and Aims: Diabetic macroangiopathy such as myocardial infarction, angina pectoris and cerebrovascular diseases are the major causes of mortality in diabetic patients. Regarding to the development of cardiovascular diseases, the presence of metabolic syndrome is an important factor because many of its components are well-accepted cardiovascular risk factors. Resistin, an adipocyte-secreted cytokines, induces insulin resistance in mice. Previous reports suggest that serum levels of resistin are increased in obese and insulin-resistant rodents and humans. Furthermore, resistin may be a link between metabolic signals and atherosclerosis in non-diabetic subjects. The objectives of this study were to explore the role of resistin in the relationship between metabolic syndrome and diabetic macroangiopathy in Japanese type 2 diabetic patients.
Materials and Methods: A total of 403 type 2 diabetic outpatients were enrolled in this study. Resistin and leptin were measured by an enzyme-linked immunoassay kit. Metabolic syndrome was determined by the criteria of NCEP-ATP3 with a minor modification. Instead of waist circumference, we used BMI to estimate obesity, because there were few patients in Japan who satisfied the original criteria. Statistical analyses were performed using the program SPSS (SPSS, Chicago, IL, USA). Data are presented as mean±SD.
Results: Serum resistin levels were positively associated with BMI (r=0.211 p=0.000) and leptin level (r=0.187 p=0.037), but not with age, duration of diabetes, systolic blood pressure, diastolic blood pressure, or the levels of fasting blood glucose, HbA1c, total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride, and adiponectin. Serum resistin levels were significantly higher in patients with ischemic heart events and cerebrovascular events than in those without these events (ischemic heart disease; 15.7 ± 9.9 ng/ml vs. 12.9 ± 8.9, p=0.029; cerebrovascular events; 18.7 ± 12.2

vs. 12.7 ± 8.5, p=0.000). The incidence of cardiac or cerebrovascular events were higher in patients with metabolic syndrome (p=0.022 and p=0.004, respectively), and increased in a score-dependent manner of metabolic syndrome. On the other hand, the serum resistin levels in patients with metabolic syndrome were also higher than those without metabolic syndrome (14.8 ± 9.9 vs. 12.8 ± 8.4, p=0.019, respectively). Interestingly the serum levels of resistin also tended to increase in a score-dependent manner of metabolic syndrome.
Conclusion: The relationship between diabetic macroangiopathy and metabolic syndrome in Japanese type 2 diabetic patients was confirmed in this cross-sectional study, and the serum resistin levels was associated with the score of metabolic syndrome and the prevalence of diabetic macroangiopathy. Those observations revealed the role of serum resistin in a link between metabolic syndrome and diabetic macroangiopathy. Further studies are needed to determine the exact role of resistin between the relationship of metabolic syndrome and the development of diabetic macroangiopathy.

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Metabolic syndrome in association with albuminuria, pulse wave velocity, and intima-media thickness in Japanese type 2 diabetic patients without nephropathy
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Background and Aims: Microalbuminuria is believed to be a marker of cardiovascular disease in the Caucasian, not only in diabetic subjects but also in the general population. However, little is known on the association between microalbuminuria and atherosclerosis in the non-Caucasian population. To test a hypothesis whether urinary albumin excretion rate, PWV, and IMT, i. e., surrogate preclinical cardiovascular risk markers, are linearly associated with grading of metabolic syndrome (MS) components in Japanese type 2 diabetic patients without cardiovascular disease and without macroalbuminuria.
Materials and Methods: We studied 536 Japanese patients with type 2 diabetes who had neither concomitant cardiovascular disease nor macroalbuminuria. The association of the number of metabolic syndrome components with albuminuria, PWV and IMT was analyzed by multiple regression models. MS components were defined by ATP-III criteria. Urinary albumin excretion rate was estimated by the mean level of three measurements of albumin-to-creatinine ratio.
Results: The levels of each component, except for blood glucose, increased with increasing the number of components significantly. Accordingly albuminuria, PWV and IMT increased with increasing the number of MS components (p<0.0001). Albuminuria was affected significantly by each presence of MS component, i. e., elevated blood pressure, abdominal obesity, high TG, and low HDL-cholesterol. PWV was significantly increased by elevated blood pressure, and IMT was significantly increased by elevated blood pressure and abdominal obesity. Multiple regression analysis indicated the number of MS components, age and glycosylated HbA_{1C} to be independent predictors of albuminuria, PWV and IMT.
Conclusions: Albuminuria, PWV and IMT showed progressive graded relationship with the number of MS components that reflects effect of the clustering of components. The finding strongly indicates that these are important surrogate preclinical cardiovascular markers. Because albuminuria, IMT and PWV are modifiable risk markers, because blood-pressure lowering drugs, particularly RAS blockers, effectively reduce these levels, the current observation may be useful to identify patients who are at high risk of experiencing cardiovascular disease, and may lead to new therapeutic strategies in the prevention of cardiovascular disease.

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Association of metabolic syndrome and their components with cardiovascular events in patients with type 2 diabetes mellitus. A cross sectional study in Spain
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Background and Aims: The role of cardiovascular disease on morbidity and mortality in diabetic patients has been known. Metabolic syndrome (MS) has been associated with a high incidence of cardiovascular events. The aims of the present work were 1) to evaluate the prevalance of MS in a cohort of patients with type 2 DM. ") To study the prevalence of the different components of MS; 3) to examine the possible relationship between cardiovascular events and the MS in this cohort of patients with type 2 DM.
Materials and Methods: A cross-sectional study was performed in 20 endocrine units in Spain, in which we recruited 927 type 2 diabetic patients who were admitted in specialised visits for two months. We used the NCEP-ATPIII criteria as a definition of MS. Body mass index, waist circumference, systolic and diastolic blood pressure (SBP and DBP) were measured. Lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol and tryglicerides), and HBA1c were determined. Clinical conditions (heart disease: myocardial infarction or angina; cerebrovascular disease: previous stroke; renal disease was diagnosed when serum creatinine was >1.5 mg/dl in men and >1.4 in women or when the estimated creatinine clearance was below 60 ml/min; and peripheral vascular disease).
Results: The mean age was 63 ± 10 years, 407 male and 520 female, with BMI= 30.5 ± 5.6 Kg/m2; SBP = 150 ± 10 mmHg and DBP= 86 ± 4.5 mmHg; HbA1c=7.6 ± 1.7%; Total cholesterol: 226.6 ± 40.6; LDL-C= 136 ± 34.4, HDL-cholesterol: 37.8 ± 12.8 Tryglicerides: 157 ± 102.23 mg/dl. The prevalence of MS was 72.1% (95% CI 68.3- 74.6). Hypertension was the most common component of MS (75.26%), follow by obesity (54.4%) and dyslipemia (34.41%). Only 17.2% of the patients showed only type 2 DM. 44.8% of them had two components of MS and only 10% had all the components (five). The diabetic patients with metabolic syndrome had more incidence of previous stroke (5.6% vs 0.6% p<0.05); more coronary artery disease (20% vs 10.2% P<0.001) as compared to the diabetic patients without metabolic syndrome
Conclusion: The prevalence of metabolic syndrome in our cohort of patients with type 2 DM is very high. Hypertension is the most important component with a greater aggregation as compared to the others components. The aggregation of metabolic syndrome involved more cardiovascular risk in type 2 diabetic patients.

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Is mild hyperhomocysteinemia an additional risk factor of the metabolic syndrome?
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Background and Aims: Characterized by abdominal obesity and insuline resistance, the metabolic syndrome is a cardiovascular risk factors cluster and mild hyperhomocysteinemia an independant risk factor, particularly for atheroma and thrombosis (Wald DS. BMJ 2002; 325 1202). We aimed to ascertain if hyperhomocysteinemia is associated with the metabolic syndrome.
Materials and Methods: «EPIMIL» is a prospective epidemiologic survey which began by a cross-sectionnal study of cardiovascular risk factors in a French population which then will be followed for ten years for supervision and intervention. Initial data collection, blood pressure measurement, ECG and blood samples (biology and DNA) have been performed. Homocysteine was assayed by High Performance Liquid Chromatography (Intra-assay variation <4.8%) and insuline by immuno-enzymology. For the metabolic syndrome, we used the criterias of the third report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (NCEP ATP III)(JAMA 2001; 285:2486). The study design has been reviewed by the Local Ethics Committee and performed in accordance with the Helsinki Declaration.
Results: Out of 2045 men aged from 20 to 58 years (38,6 ± 8,8 yrs), 185 (9%) have metabolic syndrome (at least they fulfilled 3 criterias) and 587 (29%) hyperhomocysteinemia (>12 µmol/L). Homocysteinemia doesn't differ with (11,4 ± 6 µmol/L) or without (10,9 ± 5) the metabolic syndrome. Homocysteine level does not correlated with the body mass index, waist and hip measurements, nor with glycaemia, HbA1c and insuline resistance indexes (insulinemia, HOMA-IR). It weakly correlates with systolic (r=0,063; p=0,005) and diastolic (r=0,082; p=0,0002) blood pressure, cholesterolemia (r=0,047; p=0,034), triglycerides (r=0,06; p=0,004) and free fatty acids (r=0,062; p=0,005) but not with HDL and LDL fractions, nor Lp(a). It's not associated with CRP us, nor micro-albuminuria but with creatinine clearance. It weakly contributes to the ten years cardiovascular events risk evaluated according to Framingham equations (r=0,049; p=0,03) or cardiovascular mortality according to European Score system (r=0,075; p=0,001).
Conclusion: In this population, mild hyperhomocysteinemia is not associated with the metabolic syndrome, nor with the insuline resistance indexes. It must be particularly considered when the absolute cardiovascular risk level of patients is high.
Support: Merck Sharp & Dohme, French Forces Health Service

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Evidence for elevated triglycerides and low HDL cholesterol as the main lipid risk factors for diabetic atherosclerosis

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Background and Aims: There is a paucity of longitudinal data on the impact of serum lipids on the future incidence of vascular events among the high risk cohort of coronary patients with diabetes. We aimed at investigating which lipid abnormality predominantly endangers diabetic coronary patients.
Material and Methods: We therefore enrolled 750 consecutive patients undergoing coronary angiography for the evaluation of coronary artery disease (CAD). At baseline, serum lipids were measured, and the incidence of fatal and non-fatal vascular events was recorded over 4 years. The following endpoints were recorded: coronary death, fatal ischemic stroke, non-fatal myocardial infarction, non-fatal stroke, and need for coronary artery bypass grafting, percutaneous coronary intervention, or non-coronary revascularisation.
Results: From our coronary patients, 272 (36.2%) had normal fasting glucose <5.6 mmol/l (NFG), 314 (41.9%) impaired fasting glucose ≥5.6 mmol/l (IFG), and 164 (21.9%) type 2 diabetes (T2DM). The incidence of vascular events significantly ($p < 0.001$) increased from subjects with NFG (14.7% [40 of 272 patients]) over patients with IFG (19.4% [61 of 314 patients]) to patients with T2DM (30.5% [50 of 164 patients]). Factor analysis revealed two factors in the lipid profiles of our patients: triglycerides, HDL cholesterol, apolipoprotein A1, and LDL particle diameter loaded high on an HDL-related factor; and total cholesterol, LDL cholesterol, and apolipoprotein B loaded high on an LDL-related factor. The HDL-related factor ($p < 0.001$) but not the LDL-related factor increased significantly from subjects with NFG over patients with IFG to patients with T2DM. In patients with T2DM, the HDL-related factor (OR 0.707 [0.514–0.971]; $p = 0.032$), but not the LDL-related factor (OR 1.159 [0.896–1.498]; $p = 0.261$) proved significantly predictive for vascular events.
Conclusion: The low HDL / high triglyceride pattern is associated with the degree of hyperglycemia and significantly predicts the future incidence of vascular events among coronary patients with T2DM. This charateristic diabetic dyslipidemia is thus the main lipid risk factor for vascular events in our diabetic coronary patients.

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Prospective analysis of vascular risk factors in Type 2 diabetic patients participating in a disease management programme (DMP) for diabetes mellitus

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Background and Aims: To analyse the outcomes of a disease management programme on the vascular risk profile of type 2 diabetic patients.
Materials and Methods: Participating physicians are provided with written practical guideline-based recommendations with respect to (1) patient education, lifestyle changes and multifactorial intervention of the vascular risk factors, (2) early detection of secondary complications, and (3) management of high-risk patients (i.e. patients with micro-/macroalbuminuria or impaired renal function). Regular visits of patients every 3 months with structured documentation of risk and intervention parameters. Data feedback and decision-support in form of Care Cards for both physician and patient by an evaluation centre located at the Diabetes Centre. Generation of Care Cards by a rule-based system (SAS version 9.1). So far inclusion of 4872 patients (pts.) from 647 physicians (primary care physicians or internal specialists). Analysis of 548 type 2 diabetic patients participating for at least 24 months in the disease management programme (age 64.7 ± 10.0 years, 53% males, diabetes duration 7.5 ± 6.3 years, intervention period 39.0 ± 16.6 months). Test for statistical significance of intraindividual differences by Wilcoxon signed-rank test for paired samples.

Results:

	0 months	39 months	p
HbA1c (%)	7.6 ± 1.5	7.3 ± 1.4	<0.0001
RRsyst (mmHg)	147 ± 19	140 ± 18	<0.0001
RRdiast (mmHg)	84 ± 10	80 ± 10	<0.0001
Total cholesterol (mg/dl)	221 ± 46	211 ± 44	<0.0001
Triglycerides (mg/dl)	208 ± 145	183 ± 114	<0.001
Stage of nephropathy:			
Normoalbuminuria	28%	52%	
Microalbuminuria	48%	25%	
Macroalbuminuria	4%	4%	
Impaired renal function	20%	19%	
Vascular risk score:			
Low	30%	46%	
Moderate	61%	50%	
High	9%	4%	

79% of patients with normoalbuminuria and normal glomerular filtration rate (GFR) at inclusion remained normoalbuminuric, the others progressed to nephropathy. 50% of the microalbuminuric pts. regressed to normoalbuminuria and 13% progressed. 54% of macroalbuminuric pts. regressed to microalbuminuria, one patient progressed. 36% of the pts. with impaired renal function (GFR <60 ml/min) improved to normal GFR.
Conclusion: In type 2 diabetic patients participating for more than two years in a DMP with target-oriented treatment approach and regular data feedback highly significant improvements of the vascular risk profile can be observed.

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The combination of total WBC count and hsCRP level may be a better parameter than each alone in predicting the metabolic syndrome and cardiovascular disease

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Background and Aims: Metabolic syndrome (MS) is associated with an increased risk of cardiovascular disease (CVD). Inflammation is closely associated with CVD. The serum levels of high sensitivity C-reactive protein (hsCRP) and total white blood cell (WBC) count, which are markers of systemic inflammation, had been correlated with the risk of CVD. But most studies using total WBC count and hsCRP as a marker of inflammation are limited by using these markers alone instead of combination of these markers. The aim of this study was to evaluate that the combination of total WBC count and hsCRP level may yield an additional information on predicting the MS and CVD in type 2 diabetic patients.
Materials and Methods: 591 (men 310, women 281, age 55.7 ± 1.3 yr, BMI 25.6 ± 3.3 kg/m^2) patients with type 2 diabetes were enrolled. The serum hsCRP concentrations, total WBC count and all the components of MS were measured. The history of CVD was asked. Patients with the history of malignancy, chronic inflammatory disease, their WBC count out of normal range were excluded. The patients who had acute infection and had a cold were excluded. The definition of CVD included coronary heart disease, stroke and/or peripheral arterial disease. ATP III definition of MS components was adopted in this study with the exception of the definition of obesity. This was defined as waist circumstance greater than 90 cm in men and greater 80 cm in women.
Results: The prevalence of MS was 75.3%. 123 patients (20.8%) had a history of CVD. The prevalence of MS (84.8% vs. 67.1%) and CVD (27% vs. 16%) were significantly higher in those subjects with a hsCRP > 1.0 mg/dl than in those subjects with a hsCRP≤ 1.0 mg/dl. According to the tertiles of the WBC count, the patients with highest WBC tertile had a significantly higher prevalence of MS (70.1% vs. 74.7% vs. 81.0%; tertile 1 vs. tertile 2 vs. tertile 3, $p<0.05$) and had a tendency to increase the prevalence of CVD (16% vs. 22% vs. 24%). Highest tertile of total WBC count and hsCRP >1.0 mg/dl had a highest prevalence of MS and CVD and lowest tertile of total WBC and hsCRP ≤ 1.0 mg/dl had a lowest prevalence of MS and CVD(table1). Total WBC count in combination with hsCRP level can be used to substratify risk of MS and CVD.
Conclusion: The combination of total WBC count and hsCRP level may yield an additional information on predicting the MS and CVD.

Clinical characteristics according to the hsCRP level and tertile of total WBC count

hsCRP	hsCRP ≤1 mg/dl	hsCRP ≤1 mg/dl	hsCRP ≤1 mg/dl	hsCRP > 1 mg/dl	hsCRP > 1 mg/dl	hsCRP > 1 mg/dl	p value
tertile of total WBC count	tertile 1	tertile 2	tertile 3	tertile 1	tertile 2	tertile 3	
N	131	109	80	66	89	117	
sex (female %)	50%	40%	45%	62%	48%	44%	0.097
age (years)	55.6 ± 10.1	54.8 ± 12.5	57.2 ± 9.9	56.0 ± 10.2	54.0 ± 12.2	56.7 ± 12.2	0.423
duration of DM (yr)	4.5 ± 5.5	5.5 ± 5.8	6.0 ± 5.7	3.6 ± 5.5	3.3 ± 5.0	5.1 ± 6.3	0.011
number of components of MS	2.12 ± 1.08	1.97 ± 1.06	2.19 ± 1.22	2.33 ± 1.11	2.51 ± 1.01	2.78 ± 1.00	<0.001
MS (%)	67.2%	67.0%	68.0%	75.8%	84.8%	89.7%	0<.001
CVD	13.1%	16.7%	20.0%	22.7%	28.1%	27.4%	0.036

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Incidence and risk factors of coronary heart disease in type 2 diabetic patients attending diabetes clinics: the DAI study

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Background and Aims: It is well established that type 2 diabetes (T2DM) carries an increased susceptibility to coronary heart disease (CHD), especially in women. However, little information is available on occurrence and risk factors of CHD in T2DM patients who are regularly seen at diabetes clinics and receive standard care
Materials and Methods: The reference population consisted of all patients visited at 201 Italian diabetes clinics between Sept 1998-June 1999. In each centre, a representative sample of the diabetic population was selected. A total of 14,431 patients from 157 centres (7211 women and 7220 men) participated in the incidence study and were followed up for 3 years. The present analysis is based on the 6025 women (aged 66 ± 9 years) and 5617 men (64 ± 9 years) who were free from macrovascular disease (CHD, stroke or intermittent claudication/amputations) at baseline. The data collected included anthropometrics and lifestyle habits, clinical history, data relevant to both microvascular (nephropathy and retinopathy) and macrovascular complications, laboratory data, and pharmacological treatment (for hyperglycaemia, hypertension and dyslipidaemia). Events (myocardial infarction, ischaemic heart disease, coronary artery bypass and coronary angioplasty) were ascertained by an *ad hoc* committee; results were analysed by Cox proportional hazards models.
Results: The age-standardised incidence rate of CHD was 2.4 (per 100 person-years) [95% CI 2.1–2.8] in women and 2.8 [95% CI 2.4–3.1] in men (hazard ratio [HR] for women=0.90 [0.78–1.04]). In women, independent predictors of first CHD event (n=377, of which 107 major) were: age at visit (HR=1.20 [1.05–1.38] for each 10 years), serum triglycerides (HR=1.31 [1.04–1.66]), serum total cholesterol on treatment (HR=1.70 [1.04–2.79]), insulin therapy (HR=1.58 [1.02–2.43]) and presence of microvascular complications (HR=1.47 [1.18–1.83]). In men, independent predictors of first CHD event (n=368, of which 172 major) were: diabetes duration (HR=1.06 [1.01–1.11] for each 3 years), HbA_{1c} (HR=1.11 [1.01–1.21] for each 20% increment above centre-based upper limit), and anti-hypertensive treatment irrespective of blood pressure values (HR=1.63 [1.03–2.59]).
Conclusion: In T2DM patients attending diabetes clinics, incident CHD is similar in women and men. However, the pattern of CHD risk factors is distinct for the two genders: dyslipidaemia, insulin therapy and microvascular complications stand out in women, glycaemic control and hypertension predominate in men. These findings describe the natural history of treated T2DM and may have therapeutic implications.

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The problem concerning aspirin resistance in type 2 diabetic and non-diabetic stroke patients

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Background and Aims: The role of acetylsalicylic acid (aspirin) in the secondary prevention of vascular events is well established. However, the problem of aspirin resistant patients with ischemic stroke, who do not respond on therapeutic doses of aspirin, remains not fully elucidated. The aim of the study was to determine the platelet response in type 2 diabetic compared to non-diabetic stroke patients treated with acetylsalicylic acid.
Materials and Methods: We examined 64 patients with ischemic stroke or transient ischemic attacks, assessed by computed tomography and/or magnetic resonance, classified according to Classification of Cerebrovascular Diseases III by the National Institute of Neurological Disorders and Stroke Ad Hoc Committee, with symptoms lasting less than 72 hours, receiving the routine dose of acetylsalicylic acid (150 mg daily). Group A -22 type 2 diabetic patients (15 M: 7 F, 4 patients with retinopathy, mean age: 57,2 ± 7,5 years, median time of diabetes duration: 2,5 (0,04–20) years, median HbA_{1c}: 6,3 (5,5–8,8) %, 5 patients treated with insulin, 11 patients with oral blood glucose lowering agents, 4 patients with insulin and oral blood glucose lowering agents, 2 patients with diet alone) and group B - 42 non-diabetic patients (26M: 16F, mean age: 56,2 ± 11,3 years) were studied. The control group consisted of 37 matched healthy volunteers (22M: 15F, mean age 55 ± 13,9 years), who received the same dose of acetylsalicylic acid (150 mg daily) during 10 days, as the studied patients. Patients and healthy volunteers were included to our study after giving their written informed consent according to the protocol confirmed by Bioethics Committee at the Medical University of Lodz (Poland). Platelet reactivity was assessed using platelet function analyzer (PFA-100 ^{TM}) with collagen/ epinephrine and collagen/ADP cartridges to measure aperture closure time and whole blood platelet aggregometry with 0,5 mM arachidonic acid - on the first day and after 10 days of an intake 150 mg acetylsalicylic acid in all the studied groups. Aspirin resistance was defined as the lack of the prolongation of collagen/epinephrine closure time ≤ 150 s (PFA-100^{TM} method) and/or the lack of completely inhibition of arachidonic acid - induced whole blood aggregation.
Results: In group A - 6 patients (27,3%) and 17 patients (40,5%) in group B were aspirin resistant or have the diminished response to ASA; these differences were not significant. In the control group - 3 (8,1%) persons were aspirin resistant (PFA -100^{TM} method). Laboratory assessed diminished or absent response to therapeutic doses of aspirin in both groups of patients corresponded with the clinical status and the severity of stroke (clinical worsening, computed tomography signs progression, death). Modification of antiplatelet or antithrombotic treatment in all aspirin resistant patients was necessary.
Conclusion: Our study may suggest that, the platelet reactivity monitoring during aspirin treatment may select aspirin resistant type 2 diabetic and non- diabetic stroke patients, who require individually modified antiplatelet or antithrombotic treatment in order to prevent a recurrent stroke.
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AGEs: basic mechanisms and measurement

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Advanced glycation end-product causes tolerance in immune cells with a down regulation of cell-based responses

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Background and Aims: Non-enzymatic glycation of proteins, and formation of advanced glycation end-products (AGEs), is a widely recognised contributory factor to the complications of diabetes mellitus. Whilst the role of AGEs in the pathogenesis of diabetes has been widely studied, direct effects of AGEs on cells of the immune system is a newly developing area of research. We have previously shown that the AGE methylglyoxal derived hydroimidazolone (MG-H), which is widely found *in vivo*, promotes development of the antigen-presenting dendritic cell (DC) *in vitro*, but that these cells fail to up regulate maturation markers, and lose capacity to stimulate primary T cell responses. The aim of the present study was to further characterise the effect of MG-H by assessing any change in the cytokine profiles of both DC, and the T cells they stimulate.
Materials and Methods: Normal venous blood was taken after informed consent, and the peripheral blood mononuclear cells separated by density centrifugation. These cells were cultured with MG-H-peptide, peptide alone, or buffer, then treated with fluorescently conjugated antibodies against surface molecules and intracellular cytokines. Levels of antibody staining were determined using flow cytometry.
Results: After a short incubation with MG-H (2.5–4 hrs) DC showed an increase in mitogen-stimulated IL-10 (%IL-10, buffer/MG-H: 0 ± 4.6/ 30.3 ± 6.9 [p = 0.021]), and T cells showed a substantial loss of stimulated IFN-γ and TNF-α production (e. g. %IFN-γ, $CD4^+$: buffer/MG-H, 27.8 ± 6.1/ 8.5 ± 4.8 [p = 0.038], $CD8^+$: 35.9 ± 3.5 / 11.47 ± 4.8 [p = 0.003]). Following 24 hr culture with MG-H, reduced T cell production of IFN-γ and TNF-α was no longer apparent, but the $CD4^+$ population displayed increases in IL-4 and IL-10 (%IL-4: buffer / MG-H: -3.4 ± 1.4 / 10.2 ± 5.7 [p = 0.048], no. IL-10^+: 8.7 ± 3.6 / 28.1 ± 8.0 [p = 0.033]). Also after 24 hrs, DC IL-10 had returned to control levels, but the cells were expressing less of the inhibitory receptor ILT-3 (% buffer / MG-H: 8.0 ± 1.6 / −11.4 ± 6.0 [p = 0.035]). Changes described above were not seen when cells were cultured with the peptide control.
Conclusion: The biologically relevant AGE, MG-H, has a profound effect on both DCs and the T cells they stimulate. We have shown that at early time points after contact with MG-H, DCs increased production of the immunosuppressive cytokine IL-10 in response to a stimulus, and in turn caused a dramatic reduction in the amount of cytotoxic cytokines, IFN-γ and TNF-α, released by T cells. After a longer time in culture with MG-H, the cytokine profile of T cells was skewed towards a toleragenic bias, with the $CD4^+$ ('helper') cells producing more of the growth factor IL-4 and the suppressor IL-10. The mechanism of action of MG-H would not seem to follow a classical route of tolerance via immature 'toleragenic DC' expressing more ILT-3, as this molecule was decreased in our system. These data suggest that contact with MG-H can cause a novel toleragenic state in immune cells that overrides the natural 'killer' response to mitogenic stimuli. This could have serious consequences *in vivo* for diabetic patients, reducing their ability to fight infection.
Work supported by the Medical Research Council and the North West London Hospitals NHS Trust

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The in vivo transcriptional regulation of the receptor for advanced glycation end-products in response to inflammatory stimuli

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Background and Aims: The receptor for advanced glycation end-products (AGER) has been reported to trigger intracellular responses associated with the pathogenesis of diabetic vascular disease. AGER is normally expressed at low levels in most cell types, however, AGER is reportedly upregulated in response to inflammatory stimuli. The molecular basis for its in vivo upregulation is not clearly defined. The aim of this study was to evaluate whether the chromatin fine structure of the −10 kb to +10 kb region surrounding the +1 start site of AGER was affected by lipopolysaccharide (LPS) stimulation, to allow identification of specific regulatory regions that control AGER expression.
Materials and Methods: The dynamics of in vivo chromatin fine structure alterations in response to LPS stimulation was examined by DNase I hypersensitivity (DH) in the human microvascular cell line, HMEC-1, and the hepatocytic cell line, Hep G2. AGER mRNA levels/ mRNA stability were also investigated using real-time PCR.
Results: Analysis of active chromosomal regions surrounding AGER identified a number of DH sites. The DH pattern was cell specific: in Hep G2 cells, sites were detected at −7250, +5450, +5850, +7150, +8150 bp whilst in HMEC-1 cells, sites were detected at +1, +5850, +8350 and +9350 bp relative to the +1 start site of AGER. There was no change in DH pattern in response to LPS. Endogenous levels of AGER mRNA did not change in response to LPS and AGER mRNA stability was unaffected (t1/2 = 19 hours).
Conclusion: A number of DH sites were identified in the vicinity of AGER that may be involved in the transcriptional regulation of AGER. No change in DH site pattern was detected nor were mRNA levels/ stability affected by LPS stimulation. These data suggest that transcriptional regulation of AGER is not affected by inflammatory stimuli.
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Food advanced glycation endproducts (AGE) acutely induce vascular dysfunction in patients with type 2 diabetes mellitus (T2DM), an effect reduced by benfotiamine

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Background and Aims: In patients with T2DM an AGE-rich diet can induce after 2–6 weeks persistent increases in mediators linked to vascular dysfunction (e. g. TNFα, VCAM-1). Benfotiamine (BT), the liposoluble derivative of vitamin B_1, blocks several pathways common to hyperglycaemia- and AGE-induced endothelial dysfunction. It remained unknown whether AGEs of a regular mixed meal can acutely induce vascular dysfunction in T2DM and whether this potentially angiopathic effect can be prevented by BT.
Materials and Methods: Therefore, we investigated sixteen T2DM inpatients (age: 57.9 ± 7.9 years, HbA1c: 9.5 ± 2.1%, without acute cardiovascular events within the previous 6 months) who received a standard diabetes diet for 9 days. On day 4 and 6, the acute effects of a high AGE or a low AGE meal (HAGE vs LAGE, 15.100 vs. 2.750 kU AGE) on the flow-mediated dilatation (FMD) of the brachial artery were studied in a randomized, cross-over, investigator-blinded design. FMD was assessed using high-resolution ultrasound (ATL HDI 5000, USA). In a subgroup of 11 patients the HAGE meal was repeated on day 9 after a 3-day pre-treatment with BT (Milgamma®, Wörwag, Böblingen): 3×350 mg/d on day 7 and 8, 1.050 mg on day 9, 1 h prior to the meal. FMD was assessed after an overnight fast, prior and 2, 4 and 6 h after HAGE or LAGE. Both meals had similar ingredients (580 kcal, 54 g protein, 17 g lipids, 48 g carbohydrates) and differed only by the cooking conditions (temperature and time).
Results: After HAGE, FMD decreased from 5.1 ± 1.8% at baseline to 3.6 ± 1.6*δ (2 h), 3.1 ± 1.4*δ (4 h) and 4.1 ± 1.6%*δ (6 h) (*p<0.05 vs. 0 h; δ p<0.005 vs. LAGE) and after LAGE from 5.3 ± 1.7% to 4.5 ± 1.4* (2 h), 4.2 ± 1.5* (4 h) and 5.1 ± 1.7% (6 h) (*p<0.05 vs. 0 h). BT pretreatment significantly reduced the FMD impairment induced by HAGE: 5.7 ± 2.1%– (0 h), 4.8 ± 1.8$^{\$*}$– (2 h), 4.9 ± 1.7$^{\$*}$– (4 h) and 5.5 ± 1.8%*– (6 h) ($^{\$}$p<0,05 vs. 0 h, *p<0.005 vs. HAGE without BT, –p=NS vs. LAGE). The percent changes in FMD 2, 4 and 6 hours after the meal were: −31%, −41% and −20% (HAGE), −15%, −21% and −4% (LAGE), −14%, −13% and −3% (HAGE with BT pretreatment). The endothelium-independent vasodilatation (after sublingual glyceryltrinitrate) remained unchanged.
Conclusion: In conclusion, even a single real-life AGE-rich meal can induce an acute and transient vascular dysfunction in T2DM, to a greater extent than a LAGE meal with identical ingredients. This could partly explain the increased postprandial incidence of cardiovascular events in patients with T2DM and could lead after repeated exposure to persistent vascular dysfunction. Pre-treatment with benfotiamine significantly reduced the acute postprandial vascular dysfunction of a HAGE meal to an extent comparable to that of a LAGE meal. It remains to be established whether this beneficial effect of BT is preserved after long-term dietary supplementation.

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Rosiglitazone and aminoguanidine inhibits the upregulation of RANTES induced by AGEs in human renal mesangial cells

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Background and Aims: Our previous studies showed that advanced glycosylation end products (AGEs) induced the expression of monocyte chemoattractant protein-1 (MCP-1) and initiated the onset of diabetic complications such as diabetic nephropathy (DN). RANTES (regulated upon activation, normal T cell expressed and secreted) is a member of CC chemokine family and was thought to play an important role in the early phase of DN. The present study was to investigate the effect of AGEs on the expression of RANTES in cultured human renal mesangial cells (HRMCs) and the effect of rosiglitazone and aminoguanidine on the expression of RANTES in AGEs induced HRMCs.
Materials and Methods: AGE-BSA was prepared by incubation of bovine serum albumin (BSA) with glucose at 37 °C for 12 weeks. HRMCs were incubated with serum-free medium in the presence or absence of AGE-BSA, aminoguanidine and rosiglitazone. RANTES mRNA was analysis by semi-quantity RT-PCR. The concentration of RANTES in the cultured supernatant was quantified by using ELISA. The values of RANTES mRNA were standardized by beta-actin. Data were expressed as the mean±standard deviation of at least three independent experiments. Comparisons among groups were done by one-way ANOVA.
Results: 1. After incubation of HRMCs with AGE-BSA for 48 hours, both mRNA and protein expression for RANTES was significantly increased compared to control group ($p<0.05$, 0.01, respectively). The increasing of RANTES expression was in dose and time dependent manner. 2. Both rosiglitazone and aminoguanidine significantly inhibited the expression of RANTES induced by AGE-BSA ($p<0.05$, 0.01, respectively).
Conclusion: AGEs is a potential toxin to induce expression of RANTES in HRMCs, which could be inhibited by rosiglitazone and aminoguanidine.

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Evidence for the direct inhibition of glycation by the biguanide metformin

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Background and Aims: It has been well established that guanide derivatives such as aminoguanidine have the ability to directly inhibit glycation. It has also been well-established that (1) covalently glycosylated Hb (A1c) is an acceptable surrogate marker for glycemic control and (2) metformin Rx significantly lowers HbA1c in clinical trials. The question therfore remains as to how much of metformin's effects are direct [upon glycation] and indirect [upon glycemic control.]
Materials and Methods: 921 patients were exposed in two 24 week clinical trials. The first compared metformin Rx with placebo in patients with dietary failure. The second compared metformin vs glybenclamide vs metformin + glybenclamide in patients with sulfonylurea failure. Metformin patients were pooled and linear regression models for both control and metformin patients performed expressing dHbA1c as a linear „f(dFBS)+ f(d2 hr_pc)+C" in both studies. Assuming no external effects on glycation occurred then one ought anticipate the d(HbA1c) to be nul when d(fbs) and d(2 hr_pc) were unchanged (and, therefore, the constant term, „C", should also be nul.) Finally, the overall mean treatment difference of metformin vs glybenclamide dHbA1c was evaluated because the overall EOT differences in FBS and 2 hr_PC were essentially nul.
Results: The mean treatment difference in C (dHbA1c intercept for unchanged glycemia) was -0.73%. The overall mean treatment difference in HbA1c for essentially unchanged glycemia across the two monotherapy groups in the sulfonylurea-failure population was -0.38% (99%CI (-0.10 to -0.66%).
Conclusion: Metformin appears to have a direct effect on glycation as evidenced by about a mean (absolute) range of -0.4 to -0.7% reduction in intra-erythrocyte covalent glycosylation (HbA1c) over a 24 week period.

Linear Regression Analysis (Pooled n=921)

Population	Baseline A1c	SD (A1c)	C
Control	7.71	2.75	+0.19%
Metformin	8.14	2.74	–0.54%
MTD			–0.73

Primary Efficacy Analysis in SFU Failure (n=419)

Descriptor	Baseline A1c	d(HbA1c) (MTD)	d(FBS)	d(2 hr_PC)
Metformin Monotherapy	8.28+/– 2.17%	-0.38+/- 1.58%	–0.02+/– 3.96 mM/L	–0.24 +/– 4.68 mM/L
99% CI (for MTD)		-0.10 to -0.66%		

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Assessment of catabolism of glycated and glycoxidized LDL *in vivo*: insights from small animal positron emission tomography (PET) studies

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Non-enzymatic glycation/glycoxidation of LDL is considered to play an important role in the initiation and acceleration of atherosclerosis. However, data concerning the role of circulating glycated (glycLDL) or glycoxidized LDL (glycoxLDL) in the atherogenic process are scarce. One reason for this is the lack of suitable sensitive and specific radiolabeling methods for direct assessment of metabolism of both glycLDL and glycoxLDL *in vivo*. We report an improved methodology for radiolabeling of apolipoprotein B-100 of native LDL (nLDL), glycLDL, and glycoxLDL with the positron emitter fluorine-18 (^{18}F) by conjugation with *N*-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB) and the use of [^{18}F]fluorobenzoylated LDL particles in dynamic positron emission tomography (PET) studies in male Wistar rats. As model reactions, nLDL were modified *in vitro* by either 20 mM glucose or 20 mM glucose/5 μM hemin. For radiolabeling, pools of chemically and biochemically well characterized human nLDL, glycLDL, and glycoxLDL were used. Preparation of [^{18}F]SFB was achieved by module-assisted synthesis within 68 min with radiochemical yields of 36 ± 2% (corrected for decay) and purity of >95%. LDL labeling with [^{18}F]SFB resulted in radiochemical yield of 30 ± 10% (corrected for decay) and specific radioactivity of 150–400 GBq/μmol. Radiolabeling of native and modified LDL using [^{18}F]SFB caused neither additional oxidative structural modifications of LDL lipids and proteins nor alteration of their biological activity and functionality *in vitro*, respectively. The method was further evaluated with respect to the uptake of [^{18}F]fluorobenzoylated native and modified LDL particles, respectively, in human hepatoma cells (HepG2), monocytes/macrophages (THP-1), and aortic endothelial cells (HAEC). Biodistribution studies in male Wistar rats revealed high *in vivo* stability for the [^{18}F]fluorobenzoylated native and modified LDL particles, respectively. The metabolic fate of [^{18}F]fluorobenzoylated nLDL, glycLDL, and glycoxLDL particles *in vivo* was delineated by dynamic PET studies using a dedicated small animal positron emission tomograph (microPET; spatial resolution of < 2 mm). Dynamic PET data demonstrated a significantly delayed catabolism of glycLDL when compared with nLDL. In contrast, glycoxLDL showed an enhanced catabolism when compared with nLDL. The *in vivo* distribution and kinetics of both native and modified LDL particles correlated well with the anatomical localization of LDL receptors, scavenger receptors, and receptors for advanced glycation end products. In conclusion, [^{18}F]SFB-labeling of LDL and the use of small animal PET provides a valuable tool to discriminate the kinetics and the metabolic fate of both glycLDL and glycoxLDL in animal models *in vivo*.

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Determination of in vivo transferrin glycation in diabetes mellitus: a novel method of quantification

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Background and Aims: Diabetes is associated with disturbances of glucose- and iron metabolism. It is not known how these are interrelated and how they can contribute to the increased oxidative stress in diabetes. We already demonstrated that in vitro glycation of transferrin (Tf) impairs its antioxidant function of sequestering iron in the safe redox-inactive form which is unable to participate in free radical reactions. In order to investigate if this hypothesis applies in vivo we developed a method to quantify serum Tf glycation and tested it in subjects with and without diabetes.
Materials and Methods: Fasting serum samples were collected from 107 consecutive diabetic patients attending the outpatient clinic (41/59% T1/T2; age 57 ± 14 years; 54/53 M/F and BMI 28.4 ± 5.2 kg/m^2) and 91 age- and sex-matched non-diabetic subjects. Apart from routine biochemistry, glycation was determined in total serum proteins and in Tf isolated from serum by immunocomplexation, by measuring fructosamine concentration using the nitro-blue tetrazolium assay adapted for micro-well plates.
Results: The assay for Tf glycation was first evaluated in pure Tf, pre-incubated for 14 days in vitro with increasing concentrations of glucose. The fructosamine content in Tf increased linearly with the concentration of glucose. Introduction of the immunocomplexation step did not affect linearity ($r = 0.999$) or sensitivity ($p = 0.21$ for the comparison of slopes with and without immunocomplexation). In pooled serum from subjects with normal (5.4%) and high (9.5%) HbA1c, Tf glycation was 8.80 ± 0.71 and 10.25 ± 0.61 µmol fructosamine/g Tf ($p < 0.001$), with within-run analytical variability (CV) of 6.91 and 6.06% and between-run CV of 3.93 and 2.36% respectively. Clinical evaluation in the whole diabetic group (HbA1c 7.4 ± 1.1%) revealed significantly higher Tf glycation (8.50 ± 1.11 versus 7.91 ± 1.09 µmol fructosamine /g Tf in the non-diabetic group, $p < 0.005$). In T1DM it was higher (9.07 ± 1.04) than in T2DM (8.16 ± 1.06 µmol fructosamine /g Tf, $p < 0.0005$). This was accompanied by lower total iron-binding capacity (352 ± 54 in T1DM versus 383 ± 71 µg/dL in T2DM, $p = 0.005$) and Tf concentration (238 ± 45 in T1DM versus 269 ± 39 mg/dL in T2DM, $p < 0.005$). Tf glycation correlated positively with HbA1C ($r = 0.297$, $p < 0.0005$), total serum protein fructosamine ($r = 0.329$, $p < 0.0005$) and fasting glycemia ($r = 0.231$, $p = 0.001$), and it tended to be higher in case of lower Tf concentrations ($r = –0.126$, $p = 0.076$).
Conclusion: The adapted nitro-blue-tetrazolium assay combined with immunocomplexation of serum Tf is suitable to detect differences in in vivo Tf glycation between non-diabetic, type 1 and Type 2 diabetic subjects. This method can be applied to investigate the consequences of glycation on functional stability, antioxidant function and iron-binding capacity of Tf.

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Oxidative stress in patients with metabolic syndrome: relation to dysglycemia

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Background and Aims: There is a growing evidence that oxidative stress may have significant contribution to development of insulin resistance, type 2 diabetes mellitus (T2D) and associated vascular complications. The aim of this study was to evaluate the grade of pro/antioxidative dysbalance in patients with metabolic syndrome (MS) against the background of normal glucose tolerance and T2D.
Materials and Methods: The study was carried out in 291 patients (F/M: 242/49) with MS diagnosed using the WHO definition. According to the present of T2D the subjects were divided in 2 group: group A (diabetic patients) and group B (non-diabetic patients). Group A consisted of 243 patients (age: 54.24 ± 0.47 years; BMI: 31.89 ± 0.39 kg/m^2; WHR: 0.90 ± 0.01; diabetes duration: 7.8 ± 0.6 years; fasting blood glucose levels: 7.75 ± 0.15 mmol/l; HbA_{1c}: 7.8 ± 0.18%) and group B, respectively, 48 patients (age: 53.1 ± 0.58 years; BMI: 33.63 ± 1.86 kg/m^2; WHR: 0.94 ± 0.03; fasting blood glucose levels: 5.12 ± 0.18 mmol/l; HbA_{1c}: 5.4 ± 0.11%). 21 healthy volunteers matched for age and gender served as controls. Serum lipid peroxidation products, i. e., oxidative stress markers (conjugated dienes - CD, trienes - CT, oxidienes - CO, and malondialdehyde - MDA, measured as thiobarbituric acid reacting species), total cholesterol (TC), triglyceride (TG) and NEFA were determined by spectrophotometry. Data are presented as mean±SEM.
Results: No differences in serum NEFA and early lipid peroxidation products between group A and group B were found (NEFA: 3.06 ± 0.21 vs 3.17 ± 0.26 mmol/l; CD: 0.75 ± 0.03 vs 0.99 ± 0.12 mmol/l; CT: 0.80 ± 0.07 vs 0.88 ± 0.06 mmol/l; CO: 0.49 ± 0.04 vs 0.53 ± 0.04 mmol/l). But serum TG, TC and MDA levels in group A were significantly higher than in group B (TG: 3.77 ± 0.08 vs 2.31 ± 0.12 mmol/l, $p<0.001$; TC: 6.88 ± 0.07 vs 5.76 ± 0.11 mmol/l, $p<0.001$; MDA: 2.96 ± 0.09 vs 2.00 ± 0.14 mmol/l, $p<0.001$). Highly significant ($p<0.001$) increase in all measured biochemical parameters was diagnosed in group A and group B compared to controls.
Conclusion: Our results demonstrated oxidative stress in patients with MS both against the background of normal glucose tolerance and T2D. Moreover, the comparison of diabetic and non-diabetic patients with similar anthropometric (WHR, BMI) and metabolic (NEFA) insulin resistance indices revealed the causal role of hyperglycemia in the strengthened MDA formation and elevated triglyceridemia. Taking in consideration our results and reported high toxicity of lipid-derived MDA, particularly, to cardiovascular proteins and phospholipids, we suggest that more increased serum MDA levels in patients with MS at the presence of hyperglycemia may contribute to the enhanced atherogenic risk in T2D associated with MS.

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Degradation products of proteins damaged by glycation and oxidation increase markedly in experimental diabetes and are suppressed by high dose therapy with thiamine and benfotiamine

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Background and Aims: High dose therapy with thiamine and Benfotiamine are under clinical investigation for the prevention of microvascular complications of diabetes. A mechanism of action of these interventions is prevention of advanced glycation endproducts (AGEs) formation and oxidative stress – including oxidative protein damage forming methionine sulphoxide (MetSO). Proteins containing AGE and MetSO residues undergo cellular proteolysis with release and urinary excretion of AGE free adducts and MetSO. Free adducts are the major form of excretion of AGEs and hence 24 h excretion rates reflect exposure to AGE and oxidative protein damage. In this study, we determined the effects of high dose thiamine and Benfotiamine therapies on glycation and oxidative damage in experimental diabetes.
Materials and Methods: Diabetes was induced in male Sprague-Dawley rats (250 g) by injection i.v. with 55 mg/kg STZ and body weight and moderate hyperglycaemia was stabilised by injection s.c. of 2 U of Ultralente insulin

every 2 days. Thiamine and Benfotiamine were given orally, mixed with the chow, at high dose (7 and 70 mg/kg per day) over 24 weeks to STZ diabetic and normal control rats (n = 6–13). Glycation and oxidation residues in plasma protein and free adducts in plasma filtrate and urine were determined by LC-MS/MS.
Results: The STZ diabetic rats had frank hyperglycaemia, as indicated by 5-fold increased plasma glucose concentration and 2-fold increased glycated haemoglobin HbA_1 throughout the study period. Neither thiamine nor Benfotiamine therapy improved glycaemic status of STZ diabetic rats in this study. In STZ diabetic rats, major AGE residues were increased in plasma protein (mean±SEM): CML – diabetic 0.073 ± 0.006 versus control 0.042 ± 0.004 mmol/mol lys (P<0.001); methylglyoxal-derived hydroimidazolone MG-H1 – diabetic 2.17 ± 0.12 versus control 1.29 ± 0.14 mmol/mol arg (P<0.01), and MetSO – diabetic 2.37 ± 0.20 versus control 1.17 ± 0.18 (P<0.01). All were decreased by high dose thiamine and Benfotiamine therapies except Benfotiamine failed to normalise CML residues. The most profound effect of diabetes, however, was on the 24 h excretions of AGE and MetSO free adducts. The urinary excretion of CML free adduct was increased 10-fold in STZ diabetic rats and the increase was reversed 50–60% by high dose thiamine and Benfotiamine. Urinary CML excretions (nmol/24 h) were: control 45 ± 8 and diabetic 447 ± 78 (P<0.001). The urinary excretion of MG-H1 free adduct was increased 27-fold in STZ diabetic rats and the increase was reversed 90% by thiamine and 63% by Benfotiamine in a dose dependent manner. Urinary MG-H1 excretions (nmol/24 h) were: control 94 ± 16 and diabetic 2504 ± 257 (P<0.001). The urinary excretion of MetSO free adduct was increased 3-fold in STZ diabetic rats and the increase was reversed 50% by thiamine and normalised by Benfotiamine (although plasma MetSO free adduct was increased markedly with Benfotiamine). Urinary MetSO excretions (nmol/24 h) were: control 5.2 ± 1.1 and diabetic 16.0 ± 2.4 (P<0.001).
Conclusion: There are profound increases in the urinary excretion of major AGEs and MetSO free adducts in experimental diabetes. These may be markers of tissue damage at sites of development of vascular complications and hence are novel indicators of the pathological effects of hyperglycaemia. The diabetes associated increases were reversed by thiamine and Benfotiamine.
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High glucose attenuates aspirin effects in streptozocin-diabetic rats: role of competition between nonenzymatic acetylation and glycosylatin of platelet proteins
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Background and Aims: Chronic hyperglycemia affects blood platelet reactivity in diabetes, and the preventive effects of antiplatelet drugs, such as acetylsalicylic acid (ASA), have been demonstrated. However, it has been demonstrated that in some diabetics aspirin-treatment is ineffective. The question arises whether the competition between nonenzymatic glycosylation and acetylation of platelet proteins plays a role in aspirin-resistance phenomenon.
Materials and Methods: To investigate the effectiveness of aspirin in experimental diabetes, we compared platelet reactivity and platelet protein glycation in non-diabetic and streptozotocin-diabetic rats treated with 4 mg or 40 mg ASA/kg per day p.o. for 8 weeks from the 8th day of experimentally induced diabetes. Platelet aggregation was determined at the termination of the study using whole blood impedance aggregometry with arachidonic acid (AA) or ADP as platelet agonists. Protein glycation was determined using the fructosamine method, using 1-deoxy-1-p-toluidine-D-fructose as a standard. The content of free amino groups was determined according to Sashidhar et al. using L-lysine and L-glutamic acid as standards.
Results: The dose-dependent effect of ASA "therapy" on ADP-agonized platelets was significant only in non-diabetic animals, while in diabetic rats both doses of ASA were ineffective in reducing ADP-stimulated platelet aggregation. In diabetic rats, protein glycation decreased after 2 months of ASA-treatment to levels seen in nondiabetic rats. We observed the significantly reduced amount of free amino groups in both non-diabetic and diabetic animals treated with low (from 31% in non-diabetic to 21% in diabetic rats, p <0.001) or high ASA doses (resp. from 27% to 19%, p <0.001).
Conclusion: This study demonstrates that chronic hyperglycemia interferes with preventive effects of ASA on platelet reactivity. Our data provide additional evidence to our earlier observations that aspirin ineffectiveness or 'aspirin resistance' in diabetes may be related to poor metabolic control of the disease.
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Food advanced glycation endproducts (AGE) acutely induce postprandial impairment of microvascular function in patients with type 2 diabetes mellitus (T2DM) an effect prevented by benfotiamine
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Background and Aims: Food AGE can increase after 2-6 weeks in vivo serum markers of endothelial dysfunction (e. g. TNFα, VCAM-1). Benfotiamine (BT), liposoluble vitamin B_1, blocks several pathways common to hyperglycemia- and AGE-induced endothelial dysfunction. Acute effects of food AGEs on functional parameters of microcirculation in T2DM and regulation of these effects by benfotiamine have not yet been studied.
Materials and Methods: We therefore investigated 16 inpatients with T2DM (age 57.9 ± 2.0 years, HbA1c: 9.5 ± 2.1%, 11 noninsulin-/5 insulin-treated, 5 with retinopathy, 3 with nephropathy, without acute cardiovascular events within the previous 6 months) on a standard diabetic diet for the 9 day study period. On day 4 and 6 we assessed in a randomized, investigator-blinded, cross-over design the acute effects of a high-AGE (HAGE) or a low-AGE (LAGE) meal on microvascular reactive hyperemia (RH). The HAGE and LAGE meal had the same ingredients (580 kcal, 54 g protein, 17 g lipids, 48 g carbohydrates), differences in AGE amount (HAGE vs LAGE: 15.100 vs. 2.750 kU AGE) were obtained by varying only the cooking conditions (e. g. temperature and time). In a subgroup of 11 patients the HAGE meal was repeated on day 9 after a 3-day pre-treatment with BT (Milgamma®, Woerwag, Boeblingen): 3×350 mg/d on day 7 and 8, respectively 1.050 mg on day 9, 1 h prior to the meal. RH was measured at the right hypothenar site by laser-doppler flowmetry (LEA Medizintechnik, Germany) after an overnight fast at baseline and 2, 4 and 6 hours after each meal. RH is expressed as the ratio of blood flow velocity (BFV) increase following a 4.5 min forearm ischemia (RH= post-ischemic BFV/basal BFV).
Results: RH transiently decreased after the HAGE meal from 1.4 ± 0.1 at baseline to 1.2 ± 0.1**,δ 2 h postprandially and recovered after 4 and 6 h to 1.3 ± 0.1 and 1.6 ± 0.2* respectively (**p<0.01,*p<0.5, p=NS vs. baseline, δp<0.05 vs. LAGE). RH did not decrease after the LAGE meal: 1.4 ± 0.1 at baseline and 1.5 ± 0.1 , 1.4 ± 0.1 and 1.5 ± 0.1 at 2, 4 and 6 h postprandially (=NS vs. baseline). Pre-treatment with benfotiamine abolished the effect of the HAGE meal on RH: 1.3 ± 0.1, 1.3 ± 0.1 *, 1.3 ± 0.1 and 1.6 ± 0.2 at baseline and 2, 4 and 6 hours postprandially (*p<0.001 vs. HAGE without benfotiamine, =NS vs. baseline).
Conclusion: A standardised real-life meal with a high AGE content induces a transient impairment in microvascular function. This effect can be prevented by pre-treatment with benfotiamine. Since repeated exposure to food AGE might result in persistent microvascular dysfunction, long-term effects of benfotiamine treatment in the prevention of AGE-induced vascular effects remain to be established.

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Advanced glycation end products are increased in non-diabetic patients with obstructive sleep apnea but to a lesser degree than type 2 diabetic patients
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Background and Aims: The accumulation of advanced glycation endproducts (AGEs) on tissue proteins has been implicated in the progression of age-related diseases such as diabetes mellitus and atherosclerosis. It is well known that the formation of AGEs is accelerated by hyperglycemia and

by decreased renal clearance. To determine whether oxidative stress significantly contributes to the formation of AGEs, we have compared serum AGEs concentration in patients with obstuctive sleep apnea (OSA), a condition associated with increased oxidative stress, with those in type 2 diabetic patients and healthy age-matched controls.
Materials and Methods: 119 non-diabetic OSA patients and 234 age-matched healthy controls and 134 type 2 diabetic patients were recruited. Serum AGEs were assayed by competitive ELISA using a polyclonal rabbit antisera raised against AGE-RNase.
Results: The age was similar in the 3 groups but the diabetic patients and those with OSA had significantly higher body mass index than the controls. Serum AGEs were increased in OSA subjects but to a lesser degree compared to type 2 diabetic patients (control: 3.22 ± 0.54 unit/ml; OSA: 3.68 ± 0.39, DM: 4.11 ± 0.99; ANOVA p<0.01). In the OSA subjects, serum AGEs correlated with the duration of nocturnal desaturation (r=0.21, p=0.025) and plasma total 8-isoprostane concentration, a biochemical marker of oxidative stress (r=0.22, p=0.015), but not with fasting glucose level. The association between serum AGEs and log(8-isoprostane) was independent of age, gender, BMI, smoking status and glucose on general linear model univariate analysis.
Conclusion: Serum levels of AGEs were increased in non-diabetic subjects with OSA and were associated with the severity of OSA, suggesting that increased oxidative stress can lead to an increase in the formation of AGEs even in the absence of hyperglycaemia.
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The glycation index is not an independent risk factor for microvascular complications in the DCCT when adjusted for HbA1c
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Background and Aims: The Diabetes Control and Complications Trial demonstrated that intensive therapy aimed at improved glucose control markedly reduced the risk of diabetes complications compared to conventional therapy, and that the principal determinant of risk was the lifetime history of glycemia with no threshold or breakpoint (*Diabetes*, 1995, 1996). Recently McCarter, et al., *Diabetes Care*, 2004 presented analyses of the publicly available DCCT data to support the hypothesis that the propensity for glycation is another mechanism associated with risk of complications. We present further analyses of the DCCT to show that the propensity for glycation is simply a surrogate for HbA1c.
Methods: Analysis of the risk of progression of retinopathy in the DCCT using Cox Proportional Hazards regression models.
Results: The glycation index (I) is computed as the excess HbA1c (H) above that predicted from the level of blood glucose (G)
$I = H - a - bG$
where a is the intercept and b the slope of the regression of H on G. Statistically I and H are highly correlated and thus I is simply another surrogate for HbA1c. The following table shows that stratification by I at baseline also stratifies by H.

Glycation Index Stratum	N	Mean	S.D.	HbA1c Mean	S.D.
High	470	0.1508	0.0801	10.30	1.375
Middle	470	-0.0031	0.0338	8.83	1.045
Low	469	-0.1481	0.0677	7.54	0.905

The correlation of I with H is r = 0.79. The following table shows the association between baseline HbA1c and the index I on risk of retinopathy progression, alone and together. The log glycation index is used such that there is a constant change in risk per k-fold increase in I, where arbitrarily k = 1.1 (a 10% change) is employed.

	% Change in Risk			
Proportional Hazards Model	%	95% CI	p<	R^2 (%)
1. HbA1c Alone, per 1 HbA1c % increase	49.4	38.8, 60.7	<0.0001	7.8
2. Glycation Index Alone, per 10% increase	42.9	31.3, 55.6	<0.0001	4.7
3. HbA1c and Glycation Index Together				
HbA1c, per 1 HbA1c % increase	44.6	30.5, 60.1	<0.0001	3.5
Glycation Index, per 10% increase	5.7	-6.1, 19.2	0.36	0.06

While the glycation index alone is a significant predictor (2), its effect (R^2) is less than that of the baseline HbA1c alone (1), and when adjusted for HbA1c does not significantly add to prediction of risk (3). Similar results apply to the association of mean HbA1c and glycation index values during the study with risk of complications.
Conclusion: These analyses show that the glycation index is not an independent predictor of risk of complications and that the purported effect of the glycation index on risk is wholly explained by the level of HbA1c. The glycation index should not be used to guide patient care or to modify the goals of therapy.
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Skin AGE-levels are increased in type 2 diabetes mellitus with complications
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Background and Aims: AGE formation is increased in diabetes mellitus. AGEs are thought to have a pathogenetic role in the development of diabetic complications. Several studies showed the relation between AGEs and complications in type 1 diabetes mellitus. Few studies thus far exist about the relation between AGE accumulation and microvascular and macrovascular disease in type 2 diabetic patients. In this cross-sectional analysis of an ongoing large prospective cohort of type 2 diabetic patients, we studied the relation between skin autofluorescence (AF), as a measure of tissue AGE accumulation, and the presence of microvascular or macrovascular complications.
Materials and Methods: Skin AF was measured by illumination of the lower arm with a fluorescent tube (peak intensity ~370 nm) and was calculated by correcting the mean of intensities of the emission light (420–600 nm) for the amount of reflected excitation light in the range 300–420 nm. The presence of complications was scored at the date of this measurement. We have previously validated the non-invasive skin AF measurements with AGE measurements in skin biopsies.
Results: 973 T2DM patients were classified as "no complications (35%), microvascular (27%), macrovascular (16%), or both microvascular and macrovascular (23%) complications". Mean skin AF was significantly higher in the group with both microvascular and macrovascular disease, compared to the group without complications and the group with only microvascular complications: 0.0312 (95% confidence intervaI 0.0301–0.0323) vs. 0.0257 (95% CI 0.0250–0.0265) and 0.0271 (95% CI 0.0262–0.0280), p<0.001. Skin AF in the group with only macrovascular complications (0.0291, 95% CI 0.0278–0.0303; p<0.001) was significantly higher than in the group without complications. These results could not be explained by the age-related increase in skin AF.
Conclusion: Skin autofluorescence correlates with the severity of diabetes related complications in Type 2 diabetes. Follow-up data are needed to assess whether an increase in skin autofluorescence predicts the occurrence or progression of diabetic complications.
This study was supported by a grant from the Dutch Diabetes Research Foundation (DFN 2000.00.006)

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Serum AGE-elastin derived peptides among diabetic children
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Background and Aims: Elastin is an extracellular matrix protein biopolymer, which allows organs such as lungs, skin and blood vessels to stretch repeatedly with no loss of elasticity. An increase in the amount of advanced

glycation end products (AGE) is seen in the blood of patients with diabetes mellitus. The loss of elasticity and increasing rigidity of the aging arterial wall can be attributed to loss of elastin fiber integrity and stiffening of the collagen fiber network by maintained or increased synthesis and crosslinking by the Maillard reaction.
The crosslinking as a result of the Maillard reaction, consisting in the formation and progressive modification of the structure of glucose-amine addition products (aldol condensation, Schiff base formation) resulting in the production of AGE. The purpose of the study was to measure AGE of elastin in human serum.
Materials and Methods: In the present study, we adapted an ELISA technique for the determination of AGE-elastin-derived peptides (AGE-EDP) in human sera of healthy and diabetic subjects. This test make use of human aortic elastin hydrolyzed by a chemical procedure (α-elastin) and AGE-Hemocyanin. Polyclonal sera from rabbit against AGE-Hemocyanin and from sheep against α-elastin were obtained and their specificity was tested via direct and competitive ELISA. Sera of 60 type 1 (insulin-dependent) diabetic children and 28 healthy subjects were tested.
Results: The patients with vascular complications showed significant higher levels of age, diabetes duration, systolic blood pressure (SBP), diastolic blood pressure (DBP), dose, EDP and AGE-EDP than those without vascular complications. AGE-EDP concentrations of all diabetics correlated with tryglicerides (r=0.19; p=0.04). The correlation was found between AGE-EDP and DBP in the subgroup of patients with microalbuminuria + retinopathy (r=0.94; p=0.0006). The subgroup of patients with microalbuminuria (n=19) showed correlation with age (r=0.24; p=0.008), AGE-EDP (r=0.65; p=0.0001), EDP (r=0.51; p=0.0001) and SBP (r=0.33; p=0.0003).
Conclusion: Further studies are necessary to elucidate the relationship between the serum level of AGE-elastin degradation products and diabetic vascular complications. The measurement of non-invasive markers of elastin synthesis and degradation may be useful in monitoring development and therapeutic intervention in diabetic vascular complications.

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Endothelial dysfunction: mechanisms and treatment

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Increased vascular wall endothelial nitric oxide synthase (eNOS) levels in umbilical cords from gestational diabetic women
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Background and Aims: Nitric Oxide (NO) is a key regulator of endothelial function. Hyperglycemia causes vascular damage, but its effects on NO synthesis and bioavailability are still debated. To investigate these, we obtained umbilical cords from 10 women with gestational diabetes (GD), as a model of chronic in vivo vascular cells exposure to hyperglycemia, and from 10 control women (C).
Materials and Methods: We measured: 1) eNOS protein levels (immunohistochemistry) in umbilical cord tissue 2) basal eNOS gene expression (Real Time-PCR) and protein level (Western Blot), NO production (conversion of [^{3}H]-L-arginine in [^{3}H]-L-citrulline) and nitrotyrosine levels (ELISA) in umbilical vein endothelial cells (HUVEC) cultured from GD and C cords and 3) eNOS gene expression and protein level and NO production in C-HUVEC acutely exposed (24 hours) to 25 mM glucose (HG).
Results: We observed a significant two fold increase in eNOS protein levels in umbilical cords from GD. Consistently, basal eNOS mRNA, protein content and activity were significantly greater in cultured GD-HUVEC (eNOS/GAPDH mRNA= 1.0 ± 0.1 vs 0.6 ± 0.05; eNOS protein = 0.64 ± 0.09 vs 0.37 ± 0.01 AU; NOS activity= 0.20 ± 0.02 vs 0.12 ± 0.03 pmol/mg prot.$^{-1}$/min^{-1} in GD- vs C-HUVEC, all $p<0.05$). Nitrotyrosine levels were also 3-fold increased in GD vs C. Exposure of C-HUVEC to HG induced a significant increase in eNOS mRNA, protein and activity (eNOS/GAPDH mRNA= 0.8 ± 0.05 vs 3.0 ± 0.4; eNOS protein= 0.33 ± 0.04 vs 0.74 ± 0.06 AU; NOS activity= 0.10 ± 0.03 vs 0.26 0.04 pmol/mg prot.$^{-1}$/min^{-1}, in C vs HG exposed cells, all $p<0.01$).
Conclusion: our data show that chronic hyperglycemia upregulates vascular eNOS expression in vivo in humans. This is likely to result in increased NOS activity and NO generation. However, as suggested by increased nitrotyrosine levels, in a glucose-induced pro-oxidant environment, NO is likely to react with O_2^-, thus reducing NO availability and generating potentially dangerous peroxinitrates.
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Concanavalin A, but not glycated albumin, increases subendothelial deposition of von Willebrand factor in vitro
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Background and Aims: Diabetes is associated with augmentation of prothrombogenic von Willebrand factor (vWF) content in plasma. Earlier, we have shown that high glucose and insulin do not appreciably change the deposition of vWF in subendothelial extracellular matrix (SECM) produced by cultured HUVECs. In the present work, this model was used to test conjectural effects of non-enzymatically glycated albumin (Glyc-HSA) and two lectins: concanavalin A (ConA) and wheat germ agglutinin (WGA).
Materials and Methods: The non-enzymatic glycation of albumin was performed by incubating of HSA with glucose-6-phosphate for 6 weeks, and evaluated by fluorescence measurement. 1st passage HUVECs were seeded into gelatin-coated 96-well plates and cultured for 6–7 days. HSA or Glyc-HSA (at concentrations 25, 50 and 100 μg/ml), and WGA or ConA (4, 8 and 16 μg/ml) were added 3 h after seeding. Cell viability was tested by MTT method. To determine vWF in SECM, HUVECs were detached by treatment with 0.1 M NH_4OH and the remaining material was used as a solid phase in ELISA with primary (anti-vWF) and secondary (peroxidase-conjugated) antibodies. Data were analysed using non-parametric Van der Waerden criterion.
Results: Addition of Glyc-HSA at the range of concentrations under study did not essentially influence on cell viability and vWF content in SECM (A_{490} was 0.235 vs 0.280 at none and 100 μg/ml, respectively, $p>0.05$, n=16). Cultivation in the presence of WGA led to deterioration of cell viability that constituted 74, 65 and 34% (relative to control) at 4, 8 and 16 μg/ml of the

lectin, respectively ($p<0.001$). In parallel, there was significant decrease of vWF in SECM (0.266 vs 0.128 at none and 16 µg/ml, respectively, $p<0.001$). ConA did not affect HUVECs viability, but this lectin at all concentrations reliably increased deposition of vWF (up to 152% relative to control, $p<0.001$).
Conclusion: One-week presence of glycated albumin in the medium did not change vWF content in SECM. By contrast, both tested lectins affected, though in different ways, vWF deposition. These data indicate that endothelial carbohydrate determinants and the corresponding ligands (namely, mannose/N-acetylglucosamine-specific, like hepatic mannan-binding lectin) may be involved in regulation of production and deposition of vWF.

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Effects of glucose ingestion on oxidative stress, adesion molecules and resistance arteries endothelial function
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Background and Aims: Acute hyperglycaemia promotes oxidative stress and endothelial dysfunction in vivo in men. In contrast, hyperinsulinaemia potentiates endothelium-mediated vasodilatation. When the two stimuli are physiologically elicited by glucose ingestion, and combined in different proportions according to glucose tolerance, the net effect on resistance arteries endothelial function is unknown.
Materials and Methods: Before and 180 min after the ingestion of glucose (75 g), Total Antioxidant Status (TAS), Ferric Reducing Antioxidant Power (FRAP), copper-induced LDL oxidability (LagPhase), Intercellular and Vascular Adhesion Molecules (ICAM-1 and VCAM-1) were measured in 48 subjects (16 women and 32 men, age: 53 ± 10 yrs, BMI: 28.6 ± 4.4 kg/m^2), of whom 18 had normal glucose tolerance (NT), 15 impaired glucose tolerance (IGT) and 15 diabetes (D). In a sub-group of 10 NT, 11 IGT and 10 D, the forearm vascular responses to intraarterial stepwise acetylcholine (Ach) and sodium nitroprusside (SNP) were measured (by venous occlusion plethysmography) before and 120 min after the ingestion of glucose. Conductance (blood flow/MBP*100) increments over baseline were used to express the vascular response to test drugs and the ratio (Ach/SNP) of the corresponding maximal responses was used as an index of endothelial function.
Results: In response to glucose ingestion, plasma glucose and insulin rose by 1.9 ± 0.2, 3.5 ± 0.3 and 6.2 ± 0.5 mM and by 336 ± 37, 532 ± 160, 248 ± 41 pM, in NT, IGT and D respectively. Baseline TAS was higher in D and IGT (1.00 ± 0.05 and 1.03 ± 0.07 mM) vs NT (0.79 ± 0.08 mM) while FRAP was similar in the three groups (NT: 722 ± 36, IGT:774 ± 46, D:778 ± 40 µM); both showed a 5% reduction ($p<0.01$) in response to glucose with no difference between groups for FRAP and a tendency to being more marked in D and IGT (-8% and -6% vs 2%) for TAS. Baseline ICAM-1 levels were similar in the three groups (NT:332 ± 38, IGT:263 ± 40, D:302 ± 42 ng/ml) while VCAM-1 was progressively higher as glucose tolerance deteriorated (NT:533 ± 62, IGT:672 ± 64, D:825 ± 142 ng/ml, $p<0.03$). Neither molecule changed significantly in response to glucose in any group. No difference was observed in the LagPhase at baseline (N: 70 ± 2, IGT:70 ± 2, D:67 ± 2 min) or in response to glucose. The maximal increase in forearm conductance elicited by Ach was 1099 ± 199% in NT, 995 ± 152% in IGT and 788 ± 109% in D, ($p=0.19$ by ANOVA) and 747 ± 52% in NT, 702 ± 62% in IGT and 824 ± 84% in D in response to SNP ($p=0.21$ by ANOVA). The Ach/SNP ratio was significantly depressed only in D (0.98 ± 0.12 vs 1.48 ± 0.24 in IGT and 1.46 ± 0.11 in NT, $p<0.04$). The ingestion of glucose marginally improved the response to Ach (NT: 0 ± 9, IGT: 25 ± 15, D: 25 ± 22%, p=ns), whereas it had a negative effect on the response to SNP (N:-10 ± 9, IGT:-12 ± 11, D:-6 ± 13%, $p<0.002$ by ANOVA). Thus, the Ach/SNP ratio resulted significantly improved by glucose in all groups, especially in IGT and D (NT:+20 ± 17, IGT:+55 ± 18, D:+60 ± 39%, $p<0.02$).
Conclusion: Glucose ingestion produces a mild oxidative stress that is not proportional to plasma glucose excursions, does not increase the shedding of adhesion molecules into the plasma, does not affect LDL-oxidability and, quite surprisingly, is associated with an improvement of endothelial function that counterbalances the concomitant deterioration of non-endothelium dependent vasodilatation.

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Endothelial impact of sequential lifestyle modifications in patients with metabolic syndrome
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Introduction: Patients with metabolic syndrome (MS) have a very high cardiovascular risk. Such condition is preceded by an initial promoting state known as endothelial dysfunction.
Objectives: 1- To evaluate the differential effect of cessation of smoking, diet and exercise over the endothelial function.
2- To study the diversity of responses as a function of gender, age, and diabetic state.
Material and Method: N=51 patients, aged 27 to 80 years (59,22 ± 1,97, SEM), 37/14 male/female ratio, 35 hypertensives, 23 with type 2 diabetes mellitus. Group A: n=22 smokers; group B: n=29 non-smokers; both with similar ages. Metabolic syndrome criteria: NCEP I/ATP III, sedentary lifestyle, with/without pharmacologic treatment. Randomized distribution of both groups: nutrition (A1 and B1) or exercise program (A2 and B2) during a nine-month follow-up.Anthropometric measurements: BMI (kg/m2) and waist circumference (centimetres). Glycemia, creatinine, HDL-cholesterol, LDL-cholesterol, total cholesterol, and triglycerides, evaluated by Hitachi autoanalyzer. HbA1c assessed by HPLC. Hcy: IMX Abott. Lpa: N Latex Lp(a) Reagent. Plasminogen activator inhibitor-1 (PAI-1)(ng/ml): Menarini ELISA kit. Human Monocyte Chemotactic Protein-1 (MCP-1)(pg/ml): EIA. Haemodynamic parameters: systolic and diastolic blood pressure, heart rate and pulse pressure assessed by an automatic Upper Arm Blood Pressure Monitor, OMRON MI4. Statistical analysis: Wilcoxon, Friedman-Kendall and Mann-Whitney non-parametric tests. Student parametric tests. Results were evaluated by intent-to-treat analysis.
Results: 1 – The results of initial versus final PAI-1 levels for all patients included in the study were: 44,37 ± 2.5 vs. 32,37 ± 1.92, with a statistical significance of $p<0.001$. No statistically significant differences were found in a similar evaluation with the other endothelial marker MCP-1.
2 – As a notable finding, initial PAI-1 levels of patients in the group which reduced weight were higher than for those in the group which did not ($p=0.020$). We found statistically significant differences between the initial and final PAI-1 values in patients in group A: 43,83 ± 3,35 vs 32,40 ± 3,23 (mean±standard error of mean)($p<0.020$) (fig. 3). However, it was not possible to find statistically significant differences in a similar evaluation for the other endothelial marker MCP-1. Statistically significant differences were found between the initial and final PAI-1 values in patients in group B: 44,07 ± 3,28 vs. 33,76 ± 2.64; $p<0,004$.
Conclusions: 1 – With the implementation of lifestyle modifications it is possible to reduce the endothelial aggression as evaluated by PAI-1 levels.
2 – This improvement is not related to weight reduction, therefore regular exercise itself exerts an independent beneficial effect on the endothelium.
3 – Initial PAI-1 levels of patients who do not reduce weight are lower than in patients who do so, and this could be an indicator of responsiveness.

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Effects of the addition of metformin versus pioglitazone on vascular endothelial function in type 2 diabetes patients already treated with sulfonylureas. Preliminary results
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Background and Aims: Metformin and thiazolidinediones both improve insulin resistance in patients with type 2 diabetes. Insulin resistance has been associated with endothelial dysfunction in these patients. We studied whether endothelial function improves in patients with type 2 diabetes who are already treated with sulfonylureas when metformin or pioglitazone is added to their treatment.
Materials and Methods: Diabetic patients without any history of coronary artery, cerebrovascular or peripheral vascular disease, who were already treated with sulfonylureas, were randomized into 2 groups; metformin (850 mg bd) was added in group A and pioglitazone (30 mg od) in group B for 6 months. All other treatment was maintained stable during the 6-month follow-up. Endothelium-dependent flow-mediated dilation (FMD) and endothelium-independent, nitroglycerin-mediated dilation (NMD) were assessed in all patients, using high resolution (10 MHz) linear array ultrasound in the brachial artery at baseline and at follow-up.

Results: We present the preliminary results of 20 patients who completed the follow-up (n=10 in each group). At baseline, groups A and B did not differ in age (mean±SD; 59 ± 8 vs 66 ± 8 years, respectively, p=ns), BMI, blood pressure, serum lipids, fasting glucose, HbA1c, index of insulin resistance HOMA, FMD or NMD. Following 6-month treatment, there were no significant changes in either group in BMI, blood pressure, fasting glucose, or serum lipids, apart from HDL cholesterol in group B. In group A, following 6-month treatment with metformin, there was only a small decrease in HbA1c (from 7.3 ± 0.9 to 6.8 ± 1.5%, p=0.08), while FMD did not change (from 2.00 ± 0.81% to 2.15 ± 1.61%, p=ns). In group B, following treatment with pioglitazone, there was a small decrease in HbA1c (from 7.7 ± 0.8 to 7.1 ± 1.1%, p=0.07), a significant decrease in fasting insulin (from 11.7 ± 4.3 to 8.5 ± 2.9 μU/ml, p=0.04), and in the insulin resistance index HOMA (from 4.5 ± 1.4 to 3.2 ± 1.5, p=0.008) and a significant increase in HDL cholesterol (from 49 ± 12 to 55 ± 12 mg/dl, p=0.004), while FMD significantly improved (from 1.31 ± 1.04% at baseline to 4.30 ± 3.02% at follow-up, p=0.01). NMD did not change in either group with treatment. The effects of 6-month treatment on fasting insulin, HOMA, HDL, FMD and NMD are shown in the Table.

Conclusion: In diabetic patients already treated with sulfonylureas, additional treatment with pioglitazone for 6 months improves vascular endothelial function, whereas metformin does not have a similar effect. This beneficial effect of pioglitazone may be attributed to an improvement in insulin resistance and the lipid profile of diabetic patients.

Table. Data are mean±SD.

		Baseline	6 months	*P* value
Fasting insulin (mU/L)	Metformin	10.3 ± 5.5	8.2 ± 2.8	NS
	Pioglitazone	11.7 ± 4.3	8.5 ± 2.9	0.04
HOMA	Metformin	3.6 ± 2.2	2.8 ± 1.1	NS
	Pioglitazone	4.5 ± 1.4	3.2 ± 1.5	0.008
HDL cholesterol (mg/dl)	Metformin	54 ± 12	55 ± 12	NS
	Pioglitazone	49 ± 12	55 ± 12	0.004
FMD (%)	Metformin	2.00 ± 0.81	2.15 ± 1.61	NS
	Pioglitazone	1.31 ± 1.04	4.30 ± 2.02	0.01
NMD (%)	Metformin	9.54 ± 4.93	12.42 ± 6.02	NS
	Pioglitazone	10.12 ± 3.75	9.84 ± 4.20	NS

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Rosiglitazone inhibits endothelial proliferation and angiogenesis

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Background and aims: Rosiglitazone, an insulin sensitizer, is known to offer additional beneficial effects in retarding atherosclerotic vascular lesions. Since proliferation and angiogenesis are involved in initiation and plaque instability, two critical steps in the cardiovascular events, we investigate the mechanisms of rosiglitazone on endothelial proliferation and angiogenesis.

Materials and methods: Rosiglitazone-treated human umbilical vein endothelial cells (HUVEC) were analyzed for growth rate by use of cell number counting, MTT [3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium] assay as well as ^{3}H-thymidine incorporation. Cell cycle analysis, detected by flow cytometry, cell cycle related protein, measured by Western blot, were also studied. Effects of rosiglitazone on angiogenesis were assessed by vascular endothelial growth factor (VEGF)-induced tube formation and wound healing migration. Furthermore, effects of rosiglitazone on actin stress fiber were observed under confocal microscopy.

Results: Our data show that rosiglitazone inhibits endothelial proliferation in a dose dependent manner. Rosiglitazone caused endothelial arrest at G1 phase via affected several cell cycle related proteins. Rosiglitazone markedly decreased VEGF-induced tube formation and endothelial cell migration, which might be explained by a disorganization of the actin cytoskeleton.

Conclusion: Our data suggest that both antiproliferative and antiangiogenic activities in endothelial cells might account for the greater than expected beneficial effects of rosiglitazone in the prevention and treatment of atherosclerosis.

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Rosiglitazone increases AMP activated kinase (AMPK) activity, reduces oxidative stress and extracellular-signal regulated kinases (ERKs) activity in human umbilical vein endothelial cells (HUVEC)

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Background and Aims: Hyperglycemia is a risk factor for vascular complications of diabetes and can cause long-term complications by inducing activation of protein kinase C (PKC), generation of reactive oxygen species (ROS) and accumulation of advanced glycation end products (AGEs). Furthermore, glucose mediates its adverse effects by altering the various signal transduction pathways, including extracellular signal-regulated protein kinases (ERKs). Recently it has been demonstrated that the thiazolidinediones, activators of peroxisome proliferator-activated receptor gamma (PPARgamma), have antiinflammatory, antiatherogenic, and vasodilatory effects, beyond its insulin-sensitizing action. Therefore, the aim of our study was to investigate the effects of rosiglitazone on ROS production, AMPK and ERKs activities induced by mild elevated glucose concentration in human umbilical vein endothelial cells (HUVEC).

Materials and Methods: HUVEC were grown to normal- (5 mM) or high (10 mM) glucose for 48 h. ROS production was assessed using specific fluorescent probe, Tempo-9-AC, by digital microscopy. AMPK and ERKs expression and phosphorylation were assessed by immunoblotting using specific antibodies.

Results: In cells grown at 10 mM of glucose, we observed an increased ROS production, and higher ERKs activation in comparison to 5 mM of glucose (43.12 ± 2 vs 14.85 ± 1.5 fluorescence rate, A.U., p<0,005 and pERKs/ERKs 0,84 ± 0,09 vs 0,32 ± 0,05 A.U., p<0,01, respectively), while AMPK activity was unaffected. When the cells were incubated with rosiglitazone (20 μM, 48 h), we found a significant reduction in both glucose-induced ROS production (43.12 ± 2 vs 10.01 ± 2.5 fluorescence rate, A.U., p<0,01) and ERKs phosphorylation (pERKs/ERKs 0.84 ± 0,09 vs 0,47 ± 0,06 A.U., p<0,05) and an increase of 30% of AMPK phosphorylation in comparison to unstimulated cells.

Conclusion: Our findings show for the first time that rosiglitazone upregulates AMPK activity in HUVEC, and reduces glucose-induced oxidative stress and ERKs activation. Rosiglitazone has widespread positive cellular effects: these may have important clinical implications for the prevention of diabetic complications.

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Effects of two insulin sensitizers metformin and pioglitazone on endothelial function in young women with polycystic ovary syndrome

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Purpose: Polycystic ovary syndrome (PCOS), is considered the most common endocrine disorder in women of reproductive age and is characterized by ovulatory dysfunction, with evidence of hyperandrogenism. Women with PCOS carry cardiovascular (CV) risk factors such as insulin resistance, dyslipidemia, obesity and have endothelial dysfunction, which may account for the increased CV risk. Metformin and pioglitazone are used to improve insulin resistance in women with PCOS. The aim of this study was to assess the effect of these two insulin sensitizers on endothelial function in PCOS women.

Methods: Thirty-seven young women with PCOS, non-smokers, free of CV disease, who received no hormonal or cardiovascular medications were randomized in 3 groups; group A received no treatment (n=10), group B metformin 850 mg bd (n=15) and group C pioglitazone 30 mg od (n=12) for 6 months. Endothelium-dependent flow-mediated dilation (FMD) was assessed in all women, using high resolution (10MHz) linear array ultrasound in the brachial artery at baseline and at 6 months.

Results: At baseline the 3 groups did not differ in age (mean±SD: 23.4 ± 6 vs 22.2 ± 3.6 vs 23.6 ± 5.3 years, respectively, p=ns), BMI (27.5 ± 4.8 vs 29.4 ± 6.5 vs 29.5 ± 5.1 kg/m^2, respectively, p=ns), blood pressure, serum lipids, fasting glucose and insulin, indices of insulin sensitivity, or FMD. Following treatment, FMD did not change in group A (from 5.02 ± 1.40 at baseline to 4.56 ± 1.94%, p=ns), while it significantly improved in groups B and C (from 4.09 ± 2.66 to 9.33 ± 2.19% and from 5.03 ± 2.55 to 9.23 ± 3.39%, respectively, both p<0.05). There were no changes in fasting insulin, insulin sensitivity indices or lipids in group A, whereas in group B and C, fasting insulin decreased (from 12.7 ± 5.3 to 10.2 ± 4.8 and 12.8 ± 6.0 to

8.0 ± 4.4 mU/l, respectively, both p<0.05) and fasting glucose-to-insulin ratio (index of insulin sensitivity) increased (from 6.4 ± 2.1 to 7.9 ± 2.7 and 6.8 ± 4.2 to12.8 ± 9.7 mmol/pmol/10^{-2}, respectively, both p<0.05). Furthermore, in group B, LDL-cholesterol decreased (from 119 ± 20 to 109 ± 27 mg/dl, p<0.05) and in group C, total and HDL-cholesterol increased (from 191 ± 33 to 211 ± 49 and 45 ± 7 to 53 ± 10 mg/dl,respectively, both p<0.05).
Conclusions: In young women with PCOS, treatment with either metformin or pioglitazone for 6 months has a beneficial effect on endothelial function that may be attributed to an improvement in insulin resistance and the lipid profile.

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Regulation of endothelial function

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Silymarin attenuates hypoxia and hyperglycaemic-induced DNA damage in a human umbilical vein endothelial cell line

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Background and Aims: Hyperglycaemia induced oxidative stress is known to be associated with the development of vascular complications in diabetes mellitus. The excess production of free radicals causes cellular and biochemical changes including DNA damage. In this study we evaluate the effect of both high glucose and low oxygen tension on the level of DNA damage in a human endothelial cell line. In addition the effect of the flavonoid antioxidant silymarin on DNA damage is described.
Materials and Methods: Cellular DNA damage was determined using the comet assay. Human umbilical vein endothelial cells were grown in 25 cm^2 flasks using a suspension of glucose-free Glasgow Minimum Essential Medium (GMEM) supplemented with either 5 mM or 20 mM glucose, 10% FCS, l-glutamine (200 mM), penicillin (10000 units/ml) and streptomycin (10 mg/ml). All cells were maintained at 37°C in either normoxic 5%CO_2/ 20% O_2 / 75% N_2 or hypoxic 5%CO_2/ 5% O_2 / 90% N_2 conditions. The respective growth media was supplemented with silymarin (50 µM/ml) and the DNA damage was compared to time matched controls.
Results: DNA damage was increased by 4.3% with a glucose concentration of 20 mM when compared to 5 mM (ANOVA: p=0.018; n=3) in normoxic conditions (5%CO_2/ 20% O_2 / 75%N_2) for 24 h. A further significant increase in DNA damage by 25% was evident when cells were subjected to hypoxic conditions (or 5%CO_2/ 5% O_2 / 90% N_2) for 24 h (ANOVA: p <0.001; n=3). The damage was irreversible on re-oxygenation. In the presence of silymarin there was a significant reduction in DNA damage by 7.3% compared to samples cultured in 20 mM glucose and subjected to hypoxic conditions (ANOVA: p=0.005; n=3).
Conclusion: The data derived from the comet assay provides evidence for a synergistic effect of both hyperglycaemia and hypoxia in the development of endothelial cell DNA damage. Furthermore, results suggest a cytoprotective effect of silymarin which is restricted to high glucose and low oxygen induced endothelial cell DNA damage. To our knowledge this effect has not been described before.

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Acute hyperhomocysteinemia and skin microcirculation in diabetic patients

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Background and Aims: Homocysteine (Hcy) is believed to cause vascular damage by interfering with endothelial function, platelet activation, oxidative stress, hypercoagulability and vascular smooth muscle cell proliferation. Diabetic persons are known to have endothelial dysfunction and a very high incidence of atherosclerosis. The aim of this study was to investigate the effect of acute hyperhomocysteinemia on endothelial function of skin microcirculation in diabetic persons, compared to non-diabetics.
Materials and Methods: We compared 15 type 2 diabetic persons (59.67 ± 12.06 years old, 8 males-7 females, diabetes duration 6.4 ± 4.5 years) with 14 non-diabetic ones (62.69 ± 11.69 years old, 6 males – 9 females). Skin vasosilatation was measured in arbitrary pefusion units using the Laser Doppler technique. Endothelial and non-endothelial dependent vasodilatation of the forearm skin microcirculation were evaluated with the iontophoretic administration of acetylcholine (Ach) and sodium nitroprusside (SNP) respectively, during fasting and 4 hours after p.o. administration of 0.1 mg/kg methionine (induced acute hyperhomocysteinemia).
Results: The two groups were comparable regarding age and the routine biochemical characteristics, with the exception of body mass index (BMI), waist circumference, fasting glucose, HbA1c, HOMA resistance index and fibrinogen (higher in diabetics). Fasting Hcy levels were not different between the two groups. Endothelial-dependent vasodilatation (Ach effect) did not differ between the two groups before and after acute hyperhomocysteinemia. On the contrary, endothelial-independent vasodilatation (SNP

effect) was significantly blunted in the diabetic group after acute hyperhomocysteinemia, compared to the non-diabetic one (747% vs. 1088% increase from baseline, p=0.039), after adjusting for BMI.
Conclusion: Acute hyperhomocysteinemia causes blunting of endothelial-independent vasodilatation of the skin microcirculation in diabetic persons, compared to non-diabetic ones. This finding indicates the potential contributory role of high Hcy levels on atherogenesis, especially in type 2 diabetic persons.

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The application of intravenous methacholine test in diagnosis of early functional endotheliopathy in diabetes type 1 of short and long duration
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Background and aims: The aim of work was to assess microcirculation in patients with diabetes mellitus type 1 of short and long duration, considering the presence of diabetic retinopathy, by use of intravenous methacholine test. Methacholine chloride stimulates microvascular endothelium to release nitric oxide and prostanoids and thus is a valuable tester in assessment of functional endotheliopathy.
Materials and methods: 20 persons with diabetes mellitus type 1 of short duration, without diabetic retinopathy (Group A), 18 persons with diabetes mellitus of long duration, with diabetic retinopathy (Group B) and 20 healthy persons from control group (Group C) were analysed. Values of skin blood flux in different time intervals were estimated by use of laser-Doppler flowmetry (PeriMed System 4000) during the 15-minutes infusion of methacholine chloride.
Results: The lowest relative increase of maximal perfusion was observed in persons with diabetes of long duration and diabetic retinopathy – 6,80 ± 2,15, vs. group A 11,24 ± 4,08 and group C 13,43 ± 5,00. The analysis of relative mean perfusion increase in 2, 5 and 10-minutes intervals showed lowest values in persons with diabetes of long duration, and highest in the control group. The relative increase of perfusion in diabetics of short duration, without diabetic retinopathy was statistically lower than in healthy persons in estimation of 5 minutes interval (2,81 ± 1,29 vs. 3,87 ± 1,81) and 10 minutes interval (2,16 ± 0,92 vs. 2,88 ± 1,48). Further analysis showed correlation of methacholine test parameters with other parameters such as BMI, waist circumference, waist-hip ratio and triglycerides concentration in the control group and diabetes of short duration group.
Conclusion: Differences of relative perfusion growth revealed in the study prove, that intravenous methacholine test of vascular endothelium is a new, valuable method of assessment of microcirculation in diabetic patients. The analysis of results showed decreased vascular reactivity to methacholine in persons with diabetes of short duration without retinopathy in relation to healthy persons. The new method will be very helpful in further investigations of early diabetic microangiopathy as well as in monitoring of the endotheliopathy progression in diabetes mellitus type 1.

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Evaluation of endothelial function and oxidative stress in impaired glucose regulation
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Background and Aims: As any elevation of blood glucose increases cardiovascular risk, we thought to examine whether endothelial dysfunction - an early sign of vascular damage - is present either in the fasting state or during an oral glucose tolerance test in people with impaired glucose regulation (IGR).
Materials and Methods: The study included 14 subjects with IGR (8 IGT/6 IFG, age: 49.93 ± 3.29 years; body mass index /BMI/: 26.55 ± 0,72 kg/m^2; x±SEM) and 14 matched control subjects with normal glucose tolerance (K: age: 49.28 ± 3.45 years; BMI: 26.48 ± 1.09 kg/m^2). Endothelial function was evaluated by determination of endothelium dependent (flow mediated – FMV) and independent (nitroglycerine induced – NTG) vasodilation of the brachial artery by high resolution ultrasound technique in the fasting state and one hour following the glucose load. Nitrotyrosine (NT) as an indicator of oxidative damage of the major determinant of endothelial function – nitric oxide (NO), the concentration of thiobarbituric acid reactive substances (TBARS) as an indicator of lipid peroxidation, parameters of the antioxidant defence system (total antioxidant status – TAS, ferric reducing ability of plasma – FRAP, small antioxidant molecules of the plasma) and high sensitivity C-reactive protein (hsCRP) concentrations were also determined. NT was measured by ELISA, TAS was evaluated using azino-diethylbenzthiazoline-sulphonate free radical inhibition by spectrophotometry. FRAP was measured using the rate of ferric-ferrous iron transformation with the detection of the produced ferrous-tripyridyl-s-triazine spectrophotometrically. TBARS was also determined by spectrophotometry, hsCRP by immunturbidimetry (Randox) and the small antioxidant molecules by standard laboratory methods.
Results: Fasting FMV and NTG were in the normal range in both groups with no significant difference between the groups (FMV: IGR: 11.76 ± 2.72%, K: 8.13 ± 1.89%; NTG: IGR: 20.17 ± 4.21%, K: 14.03 ± 2.55%), NT, hsCRP, TAS, FRAP and TBARS concentrations did not show any difference either. One hour following the intake of 75 g glucose, blood glucose (11.45 ± 0.25 vs. 8.86 ± 0.58 mmol/l; p<0.05), TBARS (4.87 ± 0.42 vs. 3.36 ± 0.31 mmol/l; p<0.05) and NT (194.19 ± 39.76 vs. 23.36 ± 12.28 nmol/l; p<0,05) concentrations were found to be higher in the IGR group, accompanied by a significant decrease of FMV in this group (11.76 ± 2.72% to 3.76 ± 1.39%; p<0.05), the decrease of FMV in the control group did not reach the level of significance. Significant positive correlation was observed between the NT and the blood glucose values during the glucose load and also between hsCRP and the 2 hour blood glucose values.
Conclusion: The results show that in IGR, which is considered pathogenetically to be between normal glucose regulation and diabetes mellitus, impaired endothelium dependent vasodilation and increased nitrotyrosine concentration indicating oxidative elimination of NO can be found in the post load state demonstrating a hyperglycaemia initiated increased vascular risk.

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Impact of cardiovascular status on bone mineralization.
Vascular elasticity and endothelial dysfunction as markers of osteopenia
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Introduction: Over the last few years much evidence has emerged in support of an association between osteoporosis and cardiovascular disease. Recently, statins have been proposed to induce new bone formation via an increase in bone morphogenetic protein-2. However, other conditions affected by statins, such as dyslipidaemia, vascular calcification, endothelial dysfunction and impaired nitric oxide expression, may also contribute to the cardiovascular and bone health paradigm.
Aims:
1- To analyse small and large vessel elasticity in a high cardiovascular risk population, with patients distributed into different groups according to their bone densitometry results.
2- To check whether or not there is different endothelial function in an *osteopenic population.*
Material and Methods: N=126 patients, aged 31–88 years (64,42 ± 0,87), 60/66 male/female ratio, 80,9% hypertensive, 64% with diabetes, and 19,8% smokers. Bone densitometry: accuDEXA. SCHICK Tech. INC: t-score <1: osteopenia. Metabolic parameters: creatinine, glycaemia, triglycerides, LDL, HDL, total cholesterol, assessed with HITACHI autoanalyser. HbA1c: HPLC
Extracellular matrix activity: 24-hour urinary excretion of pyridinolines/creatinine (pM/uM creatinine). HPLC Fluorimetry. JASCO FP 920. Plasminogen activator inhibitor-1 (PAI-1) (ng/ml): Menarini ELISA Von Willebrand Factor (vWF) (U/ml): Materlab ELISA kit. Large (C1) and small (C2) artery elasticity index (ml/mmHgx100), systemic vascular resistance (dyne.sec.cm5), total vascular impedance (dyne.sec.cm5). HDI/PulseWave CR-2000 Research Cardiovascular Profiling System, normal values according to age and sex (standard tables by Hypertension Diagnostics Inc. CVProfilor). Six-minute-walk: constant slope (7%) and variable speed: 0,9–6,9 mph (4,84 ± 0,31)
Statistical analysis: Mann-Whitney, Chi-Squared and Student's T tests. Spearman's rho correlation coefficient.
Results:
1. The prevalence of osteopenia was of a 31,7% (N=42). There is a significant association between osteopenia and female gender. Odds ratio=2,839, 95%CI (1,295; 6,202), p=0,009. Spearman's rho correlation coefficient r=0,236, p=0,008.
2. When comparing patients with osteopenia (N=42) to those without (n=84), we found that the former group had less elasticity in large vessels (8,88+-0,81 vs 11,46+-0,55; p=0,019) as well as in small vessels (2,34+-0,20 vs 3,49+-0,27; p=0,014). Both groups were comparable in terms of age (66,17+-1,76 vs 63,55+-0,96).
3. Subjects with osteopenia had higher extracellular matrix activity: total pyridinolines/creatinine ratio: 78,32+-8,85 vs 51,37+-3,15; p=0,002.

4. We found higher plasma levels of endothelial peptides in patients with osteopenia: vWF (270,45+-40,50 vs 195,84+-14,14; p=0,046), and PAI-1 (34,13+-2,60 vs 26,77+-1,67; p=0,026).
Conclusions:
1 – Patients with osteopenia have stiffer large and small vessels.
2 – An increment of endothelial peptides occurs in osteopenic patients.
3 – It is possible that many of the drugs considered cardiovascular protectors, such as statins, may, at the same time, be beneficial for bone cell function, exerting their action on the endothelium, the "meeting point".

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Identification of factors modulating endothelial function in healthy volunteers with different degree of insulin sensitivity
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Background and Aims: Although several abnormalities associated with insulin resistance (IR) have been implicated as causes of endothelial dysfunction, the relative importance of factors such as dyslipidemia, chronic inflammation, plasma antioxidant status, and asymmetric dimethylarginine (ADMA) levels in modulating endothelial function in apparently healthy subjects with different degrees of insulin resistance has not been evaluated.
Materials and Methods: To address this issue, the endothelial dependent vasodilatatory response to forearm ischemia (FMD) was quantified in 122 (58 M/62 F), nonsmoking, apparently healthy volunteers, divided into tertiles on the basis of their fasting plasma insulin concentration, used as a. surrogate estimate of insulin resistance. Measurements were also made of multiple anthropometric, metabolic, and hemodynamic variables. Measurements were made of plasma concentrations of high sensitivity C-reactive protein (CRP), white blood cell count (WBC), ADMA, nitrites/nitrates (NOx), cGMP, total antioxidant capacity (TAC), as well as specific determination off alpha and beta carotene, lutein, and lycopene. concentrations. Three-day food records of antioxidant intake were collected, and statistical significance of differences evaluated by ANOVA and multivariate linear regression analysis.
Results: Insulin resistant individuals had significantly higher values for BMI ($p<0.001$), concentrations of fasting plasma glucose ($p<0.001$), insulin ($p<0.001$), and triglycerides ($p<0.001$), and lover levels of high-density lipoprotein cholesterol ($p<0.001$). Insulin resistance was also associated with evidence of impaired FMD ($p<0.001$), increased inflammation (CRP $p<0.03$), greater oxidative stress (increased NOx, $p<0.005$ and decreased cGMP, $p<0.04$), reduced TAC ($p<0.002$), and lower plasma concentrations of alpha- ($p<0.005$) and beta-carotene ($p<0.001$). Dietary intake of antioxidants was similar in the three groups. Multivariate linear regression analysis indicated that fasting plasma insulin ($F=6.562$; $p<0.01$), ADMA ($F=4.151$; $p<0.03$), and alpha carotene ($F=5.859$; $p<0.02$) concentrations were independently ($p<0.05$) associated with changes in FMD, accounting for approximately 30% of the variability in FMD.
Conclusion: In conclusion, insulin resistance, assessed by compensatory hyperinsulinemia, is an independent predictor of impaired endothelial function in apparently healthy subjects. However, the origin of endothelial dysfunction in insulin resistant individuals is multi-factorial and other factors are involved. Decreased plasma antioxidant concentration and elevated ADMA levels, associated with insulin resistance, seem to have an independent impact on vascular reactivity.

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Associations among metabolic syndrome, flow-mediated-dilation and intima-media thickness in a representative sample of adult healthy population
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Background and Aims: Metabolic disease is dramatically growing all over the world representing an alarming cause of cardiovascular (CV) morbidity and mortality. In this scenario it is essential to identify the subjects at risk, to prevent overt disease. To evaluate the parameters of metabolic syndrome (MetS; NCEP ATP III criteria), along with flow-mediated dilation (FMD) and intima-media thickness (IMT), in a general healthy adult population and to study the association of metabolic abnormalities of MetS with FMD and IMT.
Materials and Methods: Among a total of 270 healthy employees of the Padova Province, representative of adult North-East Italian population, free from known metabolic abnormalities, who agreed to participate in this study, a sample of 131 subjects has been randomly selected. In all subjects anthropometric (BMI and waist, W) and blood pressure (SBP, DBP) measurements, FMD and IMT were performed along with the collection of a blood sample for the determination of: glucose (FG), insulin (FI), total (TC), HDL- (HDL), LDL- (LDL) cholesterol and triglyceride (TG), in fasting conditions. HOMA was calculated as well.
Results: Out of 131 subjects (70 females and 61 males, mean age 43 ± 0.8 yrs) 19.8% (n 26) met criteria for MetS while 80.1% (n 105) did not (C). MetS subjects compared with C were prevalently male (20/26) and, as expected, showed higher BMI (30.9 ± 0.7 vs 23.5 ± 0.4 kg/m^2; $p<0.001$), W (108 ± 2 vs 85 ± 1 cm; $p<0.001$), SBP (139 ± 2 vs 121 ± 1 mmHg; $p<0.001$), DBP (95 ± 2 vs 81 ± 1 mmHg; $p<0.001$), FG (95 ± 3 vs 87 ± 1 mg/dl; $p<0.001$), HDL (41 ± 2 vs 56 ± 1 mg/dl; $p<0.001$), TG (178 ± 14 vs 87 ± 4 mg/dl; $p<0.001$) and also higher TC (231 ± 5 vs 199 ± 3 mg/dl; $p<0.001$), LDL (154 ± 5 vs 125 ± 3 mg/dl; $p<0.001$), FI (13.4 ± 1.2 vs 8.3 ± 0.8 µU/ml; $p=0.008$), HOMA (3.2 ± 0.3 vs 1.8 ± 0.2; $p=0.002$) compared to C. The FMD was lower in MetS (7.4 ± 0.8 vs 9.3 ± 0.5%), but not statistically different, while mean IMT (0.83 ± 0.04 vs 0.65 ± 0.01 mm; $p<0.001$) and maximum IMT (1.57 ± 0.32 vs 0.98 ± 0.35 mm; $p<0.001$) were significantly higher. In the population as a whole, FMD was significantly inversely correlated with BMI, waist, SBP, DBP, TC, TG and directly with HDL. Mean IMT was significantly correlated with age, BMI, W, SBP, DBP, TC, TG, LDL, and also with FI and HOMA and negatively with HDL. No significant correlation was found in MetS subjects, probably due to the small number of cases, except between mean IMT and age.
Conclusion: The prevalence of MetS in our sample of general population is about 20%, in accordance with other epidemiological studies. Both FMD, a functional index of vascular abnormality, and IMT, an anatomic index of pre-atherosclerosis, strongly correlate with the traditional CV risk factors, but only IMT, and not FMD, correlates with the surrogate indexes of insulin-resistance in a general adult population.

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Coronary artery response to cold pressor test and microalbuminuria in type 2 diabetics and non diabetic hypertensives with angiographically normal coronary arteries and without other major risk factors
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Background and Aims: Depression of coronary artery dilation to sympathetic activation induced by a cold pressor test (CPT) and microalbuminuria are both predictive of cardiovascular events. However the relationship between these two pathophysiological processes in unknown.
Materials and Methods: Coronary artery response to CPT and urinary albumin excretion rate (UAER) have been measured in 68 normotensive type 2 diabetic patients (D2) and 77 non diabetic hypertensive patients (HT) with an abnormal ECG stress test or defects on myocardial scintiscan but with angiographically normal coronary arteries (CA). None of them was smoker or obese. Age, BMI and lipid profile were similar in the two groups. In D2, HbA1c was 6.6 ± 0.9%. Diameter of the left anterior descending CA has been measured before and after 2 minutes immersion of hands in crushed ice.
Results: A CA constriction (diameter decrease >6%) during CPT was observed in 51/68 D2 and 52/77 HT. Microalbuminuria (UAER = 30–300 mg/24 h) was found in 20/68 D2 and 7/77 HT. Microalbuminuria was found in 17/51 D2 with CA constriction, and 3/17 D2 without CA constriction, and in 4/52 HT with CA constriction and 3/22 HT without CA constriction. In the whole series, there was a negative correlation between UAER and CA diameter changes during CPT ($p = 0.005$), but no significant correlation between the increase in metabolic myocardial oxygen demand, method estimated through a rate pressure product and UAER. In D2 CA diameter changes during CPT correlated negatively with UAER ($p = 0.037$).
Conclusion: In D2 and HT with angiographically normal coronary arteries and without other major coronary risk factor, microalbuminuria is associated with a paradoxical CA constriction during sympathetic activation. These results provide further evidence for microalbuminuria as an index for endothelium dysfunction and suggest that CA endothelium dysfunction may be one of the mechanisms which might account for the poor prognosis related to microalbuminuria.

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Pathogenic mechanisms in diabetes

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Relation of enhanced ADP-ribosylation to abnormal membrane-associated processes in diabetic brain

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Background and Aims: Abnormal activity of poly(ADP-ribose)polymerase (PARP) might play a pivotal role in the pathogenesis of diabetic complications including brain dysfunction. Although it has been presumed that enhancement of poly-ADP-ribosylation within the nucleus mediates this pathological process, PARP1 and other ADP-ribosyltransferases catalyzing mono-ADP-ribosylation, which were recently shown to be localized in different cellular compartments, may also contribute to brain cell injury induced by diabetes**.** Here we investigated by using inhibitors of ADP-ribosylation, nicotinamide (NAm) and 3-aminobenzamide (3-AB)**,** whether metabolic and functional alterations in diabetic brain cells are dependent on the activation of this process in nuclei and synaptic endings.
Materials and Methods: After 5 weeks of STZ-induced diabetes (70 mg/kg of body weight, i.p.), rats were treated for 10 days with or without NAm (100 mg/kg, i.p.) or 3-AB (30 mg/kg, i.p.). ADP-ribosylation was measured by incorporation of labelled ADP-ribose from [U-^{14}C]NAD$^+$ to proteins. The membrane potentials of mitochondria (delta psi) and synaptosomes were estimated from the accumulation of radioactive permeating cation tetraphenyl phosphonium.
Results: Diabetes was found to be associated with increased oxidative stress in brain as it is evident from 1.3-fold lowering of GSH level and 2.9-fold increase in accumulation of thiobarbituric acid reactive substances vs control, $p<0.05$. Elevated ADP-ribosylation occurred in all subcellular fractions obtained from diabetic brain (respectively 14.3 ± 1.3, 64.3 ± 6.1, 110.7 ± 10.2, 286.5 ± 25.3 in diabetic vs 11.0 ± 1.1, 48.9 ± 3.9, 89.0 ± 7.5, 234.8 ± 25.8 pmol ADP-ribose/mg protein in control synaptic membranes, synaptosomes, synaptic mitochondria and nuclei, $p<0.05$). NAm and 3-AB treatments approximately with the similar ability and to the same extent in all compartments, except for synaptic membranes, caused inhibition of ADP-ribosylation. Depletion of brain NAD$^+$ and ATP contents (respectively by $27.3 \pm 1.9\%$ and $30.1 \pm 2.7\%$, $p<0.05$) defined in diabetes could be regarded as a direct consequence of free radical- and oxidant-induced increase in ADP-ribosylation since its inhibition by NAm and 3-AB efficiently downregulated oxidative stress and prevented attenuation of NAD$^+$ and ATP. Functional aspect of impaired ADP-ribosylation within synaptic terminals may have relevance to abnormal membrane-associated processes. Thus, mitochondrial transmembrane potential from isolated nerve terminals was shown to be markedly reduced (31.0%) in diabetes vs control, $p<0.05$. In addition, diabetic rats demonstrated lowering of mitochondrial ATP/ADP but higher, probably compensatory, activity of such mitochondrial respiratory chain enzyme as succinate dehydrogenase. These changes were accompanied by depolarization of synaptosomes and increased [2-^{14}C]serotonin release. Inhibition of ADP-ribosylation within the mitochondrial compartment preserved transmembrane potential and cellular respiration. NAm and 3-AB also exerted beneficial effects against diabetes-mediated depolarization of synaptic membranes.
Conclusion: Our data support a role for excessive mono- and poly-ADP-ribosylation in metabolic and functional deficits characteristic for diabetes-associated brain failures. Extranuclear ADP-ribosylating enzymes may be a drug target for the therapy of diabetic complications.

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Diabetes-associated cAMP/PKA-dependent facilitation of 5-HT release from rat brain synaptosomes: effect of nicotinamide

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Background and Aims: It has become increasingly accepted that impaired neurotransmission is implicated in diabetes-induced brain disorders. Recent advances have allowed major insights into the molecular mechanisms strongly supporting the concept of protein kinases-mediated regulation of neurotransmitter release. However, no direct association has been made between diabetes-related changes in serotonin release and intracellular signaling pathways. In the present study, we provide the evidence for the Gs protein-mediated adenylate cyclase facilitation of serotonin (5-HT) release in diabetic brain, effect which was attenuated by chronic nicotinamide (NAm) administration.
Materials and Methods: All studies were carried out after 4 weeks of STZ-induced diabetes (70 mg/kg of body weight, i.p.) in rats treated for 14 days with or without NAm (200 mg/kg, i.p.). The release of [2-^{14}C]serotonin and $^{45}Ca^{2+}$ inflow were determined in purified synaptosomes. The membrane potential was measured by the ability of synaptosomes to accumulate radioactive permeating cation tetraphenyl phosphonium.
Results: Diabetes-induced $45 \pm 3.7\%$ increase in spontaneous [2-^{14}C]serotonin release from preloaded with the mediator synaptosomes was accompanied by synaptic membranes depolarization (-41.6 ± 3.5 vs -74.0 ± 5.1 mV in control) and $36 \pm 2.9\%$ elevation of $^{45}Ca^{2+}$ inflow, $p<0.05$. Exposure of diabetic synaptosomes to protein kinase A (PKA) inhibitor, H89, as well as Ca^{2+}-free medium partially downregulated 5-HT release, indicating its dependence on increased endogenous phosphorylation and external Ca^{2+}. The facilitatory effects of cholera toxin (CTX, 1 µg/ml) or 0.01 mmol/l forskolin (activators of adenylate cyclase system) on spontaneous Ca^{2+}-dependent 5-HT release were respectively 1.5- and 1.7-fold more profound in diabetes vs control, $p<0.05$. Their actions were prevented by tetrodotoxin (TTX) and H89 that rules out the possibility of CTX and forskolin having an effect independent of the involvement of action potentials and cAMP/PKA pathway in the 5-HT release augmentation. The depolarization of nerve terminals with either 15 mmol/l KCl or 100 µmol/l 4-aminopyridine (4AP, K^+ channel inhibitor), resulted in approximately 2-fold more profound, in addition to basal, 5-HT release in diabetes vs control, $p<0.05$. In diabetes, CTX or forskolin further potentiated the release evoked by 4AP and their action was prevented by H89. 100 µM 4AP-evoked 5-HT release in the presence of forskolin exerted stimulatory effect to a similar extent as that evoked by 2 mM 4AP suggesting that the effect of forskolin on release is additive to 100 µM 4AP. However, no additive effect of the agents on membrane potential and $^{45}Ca^{2+}$ inward currents was seen that most likely suggests diabetes-induced increase in sensitivity to Ca^{2+} of exocytotic apparatus. Consistent with the involvement of action potentials in CTX- and forskolin-induced release, these compounds did not alter the release of 5-HT evoked by KCl, which action is known to be TTX-insensitive, $p<0.05$. NAm treatment initiated after 4 weeks of diabetes virtually normalized 5-HT release as well as synaptosomal response to all stimuli used.
Conclusion: The data suggest that impaired spontaneous and evoked 5-HT release associated with diabetes is, at least, dependent on G protein/cAMP/PKA-mediated facilitatory pathway in synaptic endings. NAm efficacy to prevent these changes was demonstrated.

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Differential modulation of podocalyxin-like protein (PCLP) expression by glucose concentration in human glomerular epithelial cells

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Background and Aims:Diabetic nephropathy occurs in 20 to 40% of patients with diabetes mellitus and it is considered one of the major causes of end-stage renal disease. Visceral glomerular epithelial cells or podocytes, with their foot processes and the interconnecting slit diaphragm constitute the last cellular barrier to glomerular permeability and changes of their shape are the target in many glomerular diseases. A major cell surface antiadhesin, which naturally exists on renal glomerular epithelial cells, named podocalyxin-like protein (PCLP), represents a differentiation marker for the glomerular epithelium, responsible for the formation and preservation of the foot-like processes and filtration slits of podocytes. Binding of WT1 tumor suppressor gene to conserved elements within the podocalyxin gene promoter results in potent transcriptional activation. In the adult kidney, WT1 is expressed in podocytes, suggesting an important role for WT1 in the functioning of these cells. Disruption of WT1 gene results in thickening of the glomerular basement membrane (GBM), early on in the development of renal failure and this may be one way by which WT1 plays a role in normal glomerular function.
The molecular alterations of podocytes in the presence of high glucose levels have not been fully analysed. An important issue concerns the role of WT1 in podocytes and whether it plays a role in the microfilament-based contractile apparatus of these cells. Two putative target genes of WT1, PCLP and nephrin, appear to play a role in the cytoskeletal contractile apparatus of podocytes and are involved in the regulation of the slit diaphragm. Therefore, we are mainly interested in investigating whether WT1 coordinates and/or regulates PCLP expression under physiological (low) and non-physiological (high) glucose levels.
Materials and Methods: Immortalized T-SV40 Human Glomerular Epithelial Cells (HGEC)were cultured in media containing either 5 mM or 25 mM D-glucose.

Results: HGEC continuously cultured in the presence of high (25 mM) glucose levels exhibit very low PCLP protein levels compared to HGEC continuously cultured in the presence of low (5 mM) glucose levels. The expression levels of PCLP could not be restored even after 24 weeks of culturing in 5 mM glucose. On the other hand, HGEC continuously cultured in the presence of 5 mM glucose begin exhibiting severely reduced PCLP expression levels, after being cultured in 25 mM glucose for 14 weeks. Also we investigated the expression levels of WT1. We showed that WT1 protein levels were not altered in the presence of high glucose levels. In view of the fact that p53 can alter the transcription regulatory activity of WT1 we investigated WT1 and p53 association, under low and high glucose levels. Our results showed that the association between WT1 and p53 is altered by a factor of 30% in HGEC cultured in the presence of high glucose levels compared to low (normal) glucose levels.
Conclusion: Our results indicate that the reduction of PCLP expression levels in the presence of high glucose is not due to alterations of WT1 protein levels, suggesting that chronic exposure to high glucose levels may result in irreversible down-regulation of PCLP gene expression due to alterations in the association between WT1 and p53 which may act as a negative regulator of PCLP expression, or may alter the WT1-mediated trans-activation of the PCLP gene.
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Effect of Amadori-glycated albumin on rat aortic vascular smooth muscle cells
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Background and Aims: Early Amadori-glycated serum albumin (GSA) is the predominant form of circulating glycated protein in vivo and its concentration is significantly increased in diabetes. Recent evidence suggested that it is not just an index of glycemia or precursor of advanced glycation endproducts but has biological effects on cellular functions contributing to the development of diabetic complications, such as retinopathy and nephropathy. The biological effects of GSA on macrovascular cells were poorly understood. The proliferation of vascular smooth muscle cells (VSMCs) is an essential step in the development of atherosclerosis. To investigate a potential link between GSA and diabetic macrovascular complications, we studied the effects of GSA on proliferation and gene expression of rat aortic VSMCs.
Materials and Methods: Quiescent rat aortic VSMCs were exposed to bovine serum albumin (BSA) or GSA with or without PD98059, a MAPK inhibitor. Cell proliferation was measured by MTT/XTT assay and direct counting. Gene expressions of IL-6, VCAM-1 and heat shock protein (HSP) 70 were assessed by relative quantitative RT-PCR with RNAs isolated from BSA- & GSA- treated VSMCs. To analyze signal transduction-related gene expression we used a commercially available focused oligonucleotide array (Oligo GEArray) containing 96 marker genes associated with signal transduction pathways.
Results: Rat aortic VSMCs treated with GSA (0–500 μg/mL, 48 hrs) exhibited a dose-dependent increase in proliferation that was prevented by PD98059 (25 μM), suggesting a MAPK-dependent signaling mechanism. Compared with BSA-treated cells, VSMCs treated with GSA (500 μg/mL, 4 & 24 hrs) showed a higher IL-6 and VCAM-1 gene expression but no difference in HSP 70 (Hspa1a & Hspa4) gene expression which has been reported to increase in H2O2-treated VSMCs. In focused oligonucleotide array, expression of inhibitor of apoptosis protein-1 (IAP-1, Birc3), nerve growth factor-gamma (Ngfg) and Jun genes were significantly higher in GSA-treated (500 μg/mL, 24 hrs) VSMCs than in BSA-treated VSMCs.
Conclusion: GSA induces VSMC cell proliferation through MAPK signaling. Induction of antiapoptotic proteins and strong mitogens like Ngfg by GSA might further contribute to the VSMC proliferation. In conclusion, GSA-induced VSMC cell proliferation and inflammatory cytokine gene expression may be one of the mechanisms by which hyperglycemia in diabetes accelerates atherosclerosis.

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Positive autoimmune antibodies in a diabetic pedigree with mitochondrial gene ND1 3434AG mutation
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Background and Aims: Possible association between autoimmunity and mitochondrial gene mutation in diabetes was studied in several investigations. A study found that mitochondrial gene 3243 mutation was positive in 3 of 27 Japanese ICA-positive, initially non-insulin-dependent diabetic patients. Another study revealed anti-glutamate decarboxylase (anti-GAD) antibody was positive in a 28-year-old young male with MELAS syndrome and diabetes, whose HLA typing showed DR3 and DR4. Our research further investigated the association between mitochondrial mutations and autoimmune diabetic antibodies in maternal diabetic pedigrees.
Materials and Methods: We sequenced the mitochondrial gene fragment including 16SrRNA, $tRNA^{Leu}$ gene and ND1 gene in 28 maternal diabetic pedigrees. GAD antibody and tyrosine phosphatase 2A antibody (IA-2A) were measured in subjects.
Results: In one pedigree, 2 diabetic family members were found carrying the most common nt3243 A-G mutation. Both were deaf and developed diabetes at early age, characterized by severe impaired βcell function and low BMI. GAD and IA-2A antibodies were negative in this pedigree. A new DN1 gene 3434 A-G (TAT-TGT) mutation was co-segregated with diabetes in another pedigree (10 family members including 4 diabetes, 2 IGT members and 4 normal glucose tolerance members). The proband of this pedigree was deaf. GAD antibody was positive in 1 diabetic and 2 IGT patients and 1 normal glucose tolerance family members of this pedigree. IA-2A was positive in other 2 diabetic patients. 1 normal family member showed both positive GAD antibody and IA-2A. No mitochondrial mutations and positive antibodies were found in other 26 pedigrees.
Conclusion: Mitochondrial 3434 A-G mutation causes amino acid change (Tyr-Cys) and co-segregated with diabetes in 1 pedigree, indicating that it may be diabetogenic for maternal diabetes. Furthermore, GAD and IA-2A antibodies were positive in most members of this family, implying the possibility of βcells with the mutation being susceptible to autoimmune destruction. The possible pathogenetic role of mtDNA mutations in autoimmune diabetes is worthy to be further investigated.

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H63D homozygosity confers an increased risk of diabetes in patients with haemochromatosis
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Background and Aims: Diabetes mellitus affects 20–50% of patients with haemochromatosis. One of the gene mutations responsible for haemochromatosis, C282Y, has previously been reported to be overexpressed in patients with diabetes. Our objective was to determine in our cohort if an individual's genotype for haemochromatosis affects their risk of developing diabetes.
Materials and Methods: A search of databases of patients with haemochromatosis and those with diabetes was used to identify patients with both conditions. Results of haemochromatosis genotyping studies were obtained, where available. These were compared with results from a control group of patients with haemochromatosis but no diabetes.
Results: See table below. Forty patients with both haemochromatosis and diabetes were identified. Genotypes were available in 35 of these patients. These were compared with 130 control subjects. The odds ratio for diabetes in H63D homozygous patients was 3.6 (p= 0.017, 95% C.I. 1.419 to 9.132). Patients who were compound heterozygotes had an odds ratio for diabetes of 2.3 (p= 0.09, 95% C.I. 0.9632 to 5.256). In contrast, the odds ratio for C282Y homozygous patients was 0.25 (p= 0.0005, 95% C.I. 0.1126 to 0.5356).

Genotype Differences in Haemochromatosis Patients With and Without Diabetes

Genotype	Non- diabetic n= 130	Diabetic n= 35
C282Y Homozygous	95 (73%)	14 (40%)
H63D Homozygous	13 (10%)	10 (28%)
C282Y/ H63D Heterozygous	22 (17%)	11 (31%)

Conclusion: Haemochromatosis patients in our cohort who have H63D gene mutations have an increased risk of developing diabetes, compared to other patients with haemochromatosis. These findings contrast with data from some previous studies suggesting that the C282Y genotype confers an increased risk of diabetes. The pathophysiological influences that haemochromatosis genes, in particular H63D, have on insulin resistance and diabetes warrant further study.

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Link between coronary artery disease and type 2 diabetes established from pro-apoptotic effect of patients serum on cultured human coronary myocytes

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Background and Aims: Cardiovascular complications, and especially coronary artery disease (CAD), are the first cause of death of type 2 diabetes mellitus patients. Understanding the mechanisms involved in the development of these complications is required to prevent CAD in these patients. Previous work has shown that the serum of diabetic patients with neuropathic complication promotes apoptosis of cultured neurones. We hypothesised that similar deleterious effect could link type 2 diabetes and CAD. We previously established that serum from diabetic patients increases apoptosis in human arterial smooth muscle cells. We now completed this work by studying the relation between apoptosis and the severity of coronary artery disease (CAD) on human coronary myocytes (HCM).
Materials and Methods: We collected the serum of 10 healthy volunteers (control), 17 non diabetic coronary patients and 28 type 2 diabetic patients who underwent coronary angiography after a positive screening for silent ischemia: 16 had coronary artery disease and 12 had not. Cultured HCM were incubated in the presence of 10% human serum during 48 hours. Apoptotic cells were then detected by annexin-V and TUNEL (terminal transferase nick end labelling) labelling. The analyse was performed by flow cytometry.
Results: The percentage of apoptotic nuclei assessed by TUNEL was not different between control (11.1 ± 1.2%), diabetic (11.5 ± 0.5%) and non-diabetic coronarian (12.5 ± 0.7%) subjects. In contrast, we found significantly higher apoptosis for HCM in the presence of serum from diabetic patients with CAD (19.2 ± 1%), which reflected a 64% fold increase in the number of apoptotic cells. These results were confirmed with annexin-V labelling.
Conclusion: Proapoptotic effects of serum were observed only in diabetic patients with CAD. Type 2 diabetes might induce or aggravate coronary lesions in a sub-population of diabetic patients at high risk. We will now investigate whether a link exists between the degree of apoptosis and the severity of CAD.

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B-natriuretic peptide: characterisation of a new cardiovascular biomarker in type 2 diabetic patients with and without nephropathy

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Background and Aims: B-natriuretic peptide (BNP) is a cardiac neurohormone predominantly released from the cardiac ventricle in response to left ventricular volume expansion and pressure overload. Thus it represents a new marker for myocardial stress. Type 2-diabetic patients with nephroapthy (N) show a high cardiovascular morbidity; however, there are few informations about the levels of NT-proBNP in this patient group with respect to degree of renal dysfunction, albuminuria, parameters of metabolic and blood pressure control, presence of anemia and other new risk indicators such as homocysteine and high sensitive CRP (hs).
Materials and Methods: In 279 Typ 2-diabetic patients without (n=104) and with nephropathy (n=175; defined as persistent albuminuria and/or creatinine clearance <90 ml/min) the following parameters were examined: serum concentration of NT-proBNP (Roche Diagnostics,Mannheim), hs CRP, homocysteine, lipids, Creatinine-Clearance (CCL), urine albumin excretion, hemoglobin, blood pressure (ABDM). History of macrovascular disease (CHD, stroke, peripheral artery disease, amputation, heart failure) was recorded.
Results: Patients with N. were older (65 vs 59 years) and had a longer duration of diabetes (11,5 vs 7 years) than patients witout N. Levels of NT-proBNP were higher in patients with than without N. (median 111 vs 63 ng/l; p<0,001). In patients without N. Nt-proBNP showed a weak correlation to HbA1c (spearman correlation coefficient -0,22; p= 0.02), to systolic blood pressure (0.22; p=0.03) and CCL (–0.20; p=0.03). In patients with N. NT-proBNP were strongly correlated to CCL (–0.33; p=0.008), urinary albumin excretion (0.34; p<0.0001), blood pressure values during night (not during day), hemoglobin (–0,019, p=0.008), but not to HbA1c., homocysteine or hs CRP. Patients with renal insufficiency and pesistent albuminuria showed significantly higher NT-proBNP values than patients without albuminuria irrespective of the CCL (median NT-proBNPlevels at CCL 60–90 ml/min 280 vs 91 ng/l, at CCL <60 ml/min 461 vs 101 ng/l). Patients with known history of macrovascular disease had higher Nt-proBNP levels than patients without, irrespective of kidney dysfunction.
Conclusion: The higher levels of Nt-proBNP in Type 2 diabetic patients with N. correspond to the known high risk of cardiovascular complications in this patient group. It could be shown that NT-proBNP levels are correlated to blood pressure during night and concentration of hemoglobin, indicating the clinical significance of good blood pressure control during night and treatment of anemia. According to NT-proBNP levels, patients with diabetic N. i. e. CCL <90 ml/min and persistent albuminuria, exhibit a higher myocardial load than patients with an ischemic type of N., i. e. CCL <90 ml/min without albuminuria.

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Women after gestational diabetes - does the adrenal gland play a role in the future development of type 2 diabetes?

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Background and Aims: Women who have had gestational diabetes are at greater risk of developing type 2 diabetes. Although many are insulin resistant, this along with other factors which influence insulin resistance, does not allow us to predict who will go on to develop diabetes.
Recent reports suggest that subtle abnormalities of adrenal hormones play a role in the pathogenesis of type 2 diabetes. Improved understanding of this concept may allow better methods to determine who is likely to develop diabetes in the future. In this study we aimed to look at whether baseline testosterone or the cortisol response to a glucose load (a simple measure of hypothalamic pituitary adrenal axis sensitivity) differed between women who remained euglycaemic and those who went on to develop IGT or diabetes.
Materials and Methods: We invited women in the greater Bristol area with previous gestational diabetes to attend for a visit. At this visit fasting glucose, insulin, lipids, cortisol and testosterone were taken. Two hours after a 75g glucose load further blood was taken for glucose and cortisol. Height, weight, sitting blood pressure and waist hip ratio were also taken. HOMA analysis was performed. T tests were used to compare groups, and correlation performed to look at relationships between results.
Results: 92 of 332 women contacted attended for assessment, mean age 36 (25–48 years). Mean follow up was 3 years after gestational diabetes (2 months-12 years). Six (7%) had diabetes at follow up and a new diagnosis of diabetes or of IGT was made in 7 (8%) and 25 (27%) women respectively. Comparing unaffected women with those with IGT and with diabetes, there was no difference in age, time since gestational diabetes, number of children, blood pressure, cholesterol or waist hip ratio. Participants with diabetes tended to have a higher BMI (mean 31.3 ±; SD7.33 v 27.6 ±; 5.67, p=0.06), higher triglycerides (1.4 ±; 1.1 v 1.0 ±; 0.5 mmol/l, p<0.05) and were more insulin resistant (HOMA2IR 1.8 ±; 0.98 v 1.1 ±; 0.68, p=0.009) than unaffected women. Participants with IGT had a higher BMI (31.2 ±; 8.6 v 27.6 ±; 5.67, p=0.03) and were more insulin resistant (HOMA2IR 1.9 ±; 1.3 v 1.1 ±; 0.68, p=0.005) than unaffected women.
Participants with diabetes had a higher testosterone level (2 ±; 0.7 v 1.4 ±; 0.8 mmol/l, p=0.01) and a trend towards a greater fall in cortisol after a glucose load (132 ±; 134 v 40 ±; 134 mmol/l, p=0.06) when compared to unaffected. Participants with IGT showed no difference in testosterone level (1.4 ±; 0.6 v 1.4 ±; 0.8 mmol/l, p=0.4) but had a significantly greater fall in cortisol after glucose (103 ±; 152 v 40 ±; 134 mmol/l, p=0.04) than unaffected women. Fall in cortisol or baseline testosterone did not correlate with insulin resistance, BMI, cholesterol, triglycerides, waist hip ratio or blood pressure.
Conclusion: We have found increased testosterone levels and heightened sensitivity of the hypothalamic pituitary adrenal axis to a glucose load in individuals who develop abnormal glucose tolerance after gestational diabetes. These findings are independent of factors usually associated with the development of abnormal glucose tolerance. Further research is required to determine whether these changes are a consequence of abnormal glucose tolerance or precede it. If the latter were the case, these may provide simple measures to aid in the prediction of type 2 diabetes.
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Vascular benefits of rosiglitazone and metformin compared with sulphonylurea and combination therapy

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Background and Aims: Hypertension is a major factor in the increased cardiovascular risk associated with insulin resistance (IR) and type 2 diabetes (T2D). Rosiglitazone is an insulin sensitizing oral hypoglycaemic agent with putative cardiovascular benefits including potential effects on blood pressure, vascular function and inflammation. We therefore conducted a randomised double blind study assessing the vascular and anti-hypertensive effects of Rosiglitazone based therapy compared with sulphonylurea based therapy.
Materials and Methods: 50 T2D patients on metformin (HbA1c>6.5%) received rosiglitazone (R), 8 mg daily or gliclazide (G) 160 mg daily. 24 hour ambulatory blood pressure, aortic compliance (augmentation index (Aix)), HbA1c, lipid profile, HOMA-IR, hs-CRP, oxidative stress (TBARS) and soluble markers of endothelial activation (e-Selectin, ICAM-1) were measured at baseline and at 3 months.
Results: Baseline blood pressure, vascular and all metabolic parameters were similar in both groups. Hba1c fell by a similar extent in both groups (1.09% (R) and 1.13% (G)). HOMA-IR (1.12 ± 0.3 (R) vs. 0.45 ± 0.1 (G)), hs-CRP (0.44 ± 0.4 (R) vs. 0.13 ± 0.09 ng/l (G)), e-selectin (64.5 ± 21.1 (R) vs. 22.9 ± 12.9 µmol/l(G)), ICAM-1(403 ± 61 to 208 ± 55 (R) vs. 388 ± 49 to 311 ± 43 µmol/l (G)) and Aix (19.3% (R) vs. 3.1% (G)) and TBARS all fell to a greater extent in the rosiglitazone group ($p < 0.05$). There were also significant reductions in mean 24 hour BP (131.3 / 78.9 ± 10.1 / 5.4 to 133.7 / 80.4 ± 8.8 / 5.9 (G) vs. 133.2 / 79.4 ± 9.2 / 6.4 to 126 / 75.4 ± 8.6 / 4.5 (R), $p < 0.05$) and mean arterial pressure in the rosiglitazone group (88.3 ± 3.9 to 90.3 ± 7.8 (G) vs. 88.9 ± 5.9 to 83.5 ± 4.5 (R)) compared to baseline and the gliclazide group. These BP changes correlated with the change in augmentation index ($r=0.428$, $p<0.05$)and insulin resistance ($r=0.457$, $P<0.05$).
Conclusion: Compared with glicalzide, rosiglitazone appears to exert greater vascular benefits including anti-oxidant, anti-inflammatory and blood pressure lowering properties. The anti-hepertensive effects of rosiglitazone may relate to imporved vascular function and insulin sensitivity. Outcome studies are however awaited to confirm the impact of these effects on vascular events.

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Animal models of metabolism and complications

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Impaired cholesterol absorption in transgenic mice overexpressing spermidine/spermine N1-acetyltransferase is due to reduced expression of Niemann-Pick C1 like 1 in jejunum

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Background and Aims: Cholesterol level is determined by a balance between intestinal absorption and endogenous synthesis of cholesterol. Low cholesterol absorption efficiency and increased cholesterol synthesis are associated with insulin resistance and type 2 diabetes. In this study, we investigated transgenic mice overexpressing spermidine/spermine N^1-acetyltransferase (SSAT), the key enzyme in the catabolism of polyamines. SSAT mice have improved glucose tolerance, increased insulin sensitivity and low total cholesterol level. The aim of our study was to investigate the causes for altered cholesterol metabolism in this mouse model.
Materials and Methods: Plasma total cholesterol, cholestanol, cholesterol precursors and plant sterols were measured by gas-liquid chromatography in the fasted and fed state in 4-month-old female SSAT mice. In addition, cholesterol absorption efficiency was measured with fecal dual-isotope ratio method in 6-month-old female SSAT mice. Gene expression levels in liver and jejunum were analyzed in fed 4- to 6-month-old female SSAT mice using quantitative RT-PCR.
Results: In the fasting state, plasma total cholesterol levels (1.4 ± 0.1 vs. 2.2 ± 0.2 mM, $p<0.01$) and cholesterol absorption markers (campesterol: 903 ± 106 vs. 1410 ± 180 10^2x µmol/mmol of cholesterol, $p<0.05$ and cholestanol: 167 ± 13 vs. 249 ± 17 10^2x µmol/mmol of cholesterol, $p<0.01$) were reduced in SSAT mice compared to wild-type mice. In contrast, the levels of cholesterol precursors were significantly higher in SSAT mice than in wild-type mice (squalene: 187 ± 10 vs. 98 ± 10 10^2x µmol/mmol of cholesterol, $p<0.001$, desmosterol: 156 ± 15 vs. 68 ± 9 10^2x µmol/mmol of cholesterol, $p<0.001$ and lathosterol: 149 ± 15 vs. 90 ± 10 10^2x µmol/mmol of cholesterol, $p<0.01$). Similar results were obtained in the fed state. Cholesterol absorption efficiency was significantly lower in SSAT mice than in wild-type mice (65 ± 2 vs. 74 ± 2%, $p<0.05$). Expression of genes involved in the cholesterol synthesis pathway were elevated in the liver of SSAT mice compared to wild-type mice (squalene synthase: 1.83 ± 0.09 fold, $p<0.01$, HMG-CoA reductase: 2.44 ± 0.28 fold, $p<0.01$ and 7-dehydrocholesterol reductase: 1.70 ± 0.06 fold, $p<0.01$). Of the genes involved in cholesterol absorption, Niemann-Pick C1 Like 1 (NPC1L1) was reduced in SSAT mice (0.61 ± 0.04 fold, $p<0.01$) in jejunum.
Conclusion: Our results suggest that the activation of polyamine catabolism in SSAT mice causes reduced total cholesterol level due to low cholesterol absorption which is not compensated by enhanced hepatic cholesterol synthesis. Impaired cholesterol absorption is apparently a result of reduced expression of NPC1L1 in jejunum. The identification of mechanisms via which polyamines regulate cholesterol absorption may offer a novel target for the drug development for hypercholesterolemia.
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Cilostazol down-regulates expression of NF-kappaB-mediated VCAM-1 in STZ-induced diabetes: possible role of PPARs

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Background and Aims: Cilostaozl, a specific inhibitor of cAMP phosphordiesterase, has been found to down-regulate adhesion molecules, but the underlying mechanism is not clear. Previous reports showed that nuclear factor(NF)-kappaB, maybe corporated with other transcription factors, regulates expression of vast series of genes. In the present investigation, we examined the effect of cilostazol on NF-kappaB activation and PPARs expression in aorta of STZ-induced SD rats.

Materials and Methods: Upon treated with different dose of cilostazol ($27\,mg \cdot kg^{-1} \cdot d^{-1}$ and $9\,mg \cdot kg^{-1} \cdot d^{-1}$) for 8 weeks, rats were sacrificed and aortae were stored. To confirm the DNA binding activity of NF-kappaB, we performed an electrophoresis mobility shift assay (EMSA). To determine the expression of VCAM-1 and translocation of NF-kappaB p65, we examined the content of these two proteins in cytoplasm or karyon by immunohistochemistry. Also we detected VCAM-1 mRNA expression with in situ hybridization. At last mRNA expression of PPARalpha and PPARgamma were assessed by Real-time PCR.
Results: The NF-kappaB-DNA binding activity was significantly increased in aorta of diabetic rats. Similarly, the levels of nuclear p65 increased in parallel with the upregulation of VCAM-1 and its transcripts. All of these were reversed by high dose of cilostazol ($27\,mg \cdot kg^{-1} \cdot d^{-1}$). Also, high dose of cilostazol up-regulated PPARalpha mRNA and down-regulated PPARgamma mRNA in aortae of diabetic rats.
Conclusion: It has been reported that several domains of PPARs can be phosphorylated by PKA in vitro and PKA stabilizes binding of the liganded PPARs to DNA. Furthermore, PPARs may modulate transcript expression either directly or by interaction with other signal pathway, such as AP-1, STATs and NF-kappaB, which suggests that cilostazol, may reduce VCAM-1 expression via, at least in part, changes of PPARs, and then inhibition of NF-kappaB activity. We believe that this represents a novel potential insight into the possible inhibitory mechanism of cilostazal on VCAM-1, and into the protection effects in diabetic macroangiopathy.
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Alteration of nutrient-stimulated insulin release in pancreatic islets of ω3 fatty acid-depleted rats and its correction by intravenous injection of a medium-chain triglyceride: fish oil emulsion
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Background and Aims: It was recently reported that, in both rats and human subjects, the bolus intravenous injection of a medium-chain triglyceride:fish oil emulsion (FO) increases within 60 min and for at least 24 hours the content of long-chain polyunsaturated ω3 fatty acids in cell phospholipids. The present study aimed mainly at comparing metabolic and secretory variables in pancreatic islets of control and ω3-depleted rats and at investigating the possibility of promptly correcting alterations by the injection of the FO emulsion in the latter animals.
Materials and Methods: Female fed control and ω3-depleted rats were examined. In some experiments, the ω3-depleted rats were injected intravenously 120 min before sacrifice with 1.0 ml of either the FO emulsion or a control medium-chain triglyceride:olive oil emulsion (OO). In addition to measuring glucose and insulin in plasma, protein and insulin content, utilization of D-[5-^{3}H]glucose and oxidation of D-[U-^{14}C]glucose as well as insulin release were analysed in pancreatic islets.
Results: In ω3-depleted rats, the mean plasma concentration of glucose (8.38 ± 0.88 mM) and insulin (1.39 ± 0.18 ng/ml) were slightly higher than in control animals (7.03 ± 0.41 mM and 0.93 ± 0.10 ng/ml). The protein and insulin content of the islets was comparable, however, in control and ω3-depleted rats. When compared to control rats, two metabolic anomalies were found in the islets of ω3-depleted rats. First, the relative magnitude of the increase in both D-[5-^{3}H]glucose utilization and D-[U-^{14}C]glucose oxidation, in response to a rise in D-glucose concentration from 2.8 to 8.3 and 16.7 mM was less pronounced. Second, at all D-glucose concentrations, the paired ratio between $^{14}CO_2$ output and ^{3}HOH generation was significantly lower. The absolute value for insulin release evoked by 8.3 mM D-glucose was comparable in ω3-rats injected with the OO and FO emulsion. However, relative to basal value such a release was higher in the OO than FO group. Likewise, when expressed relative to basal value, the output of insulin evoked by 2-ketoisocaproate (10 mM) or L-leucine (20 mM), as well as the enhancing action of L-glutamine (1.0 mM) upon leucine-stimulated insulin release, were all higher in the OO than FO group. The values found in the latter animals averaged $62.8 \pm 3.9\%$ and $98.6 \pm 7.3\%$ ($n = 79$) of the corresponding values found in the ω3-depleted rats injected with the OO emulsion and normal rats, respectively.
Conclusion: The ω3-depleted rats display features compatible with insulin resistance. In the islets of these rats, the two anomalies of D-glucose metabolism might well be attributable to an accelerated catabolism of circulating free fatty acids, the clearance of which is indeed increased in ω3-rats. The present secretory data also suggest that the intravenous injection of the FO emulsion corrects a perturbation otherwise prevailing in ω3-depleted rats and conceivably linked, in part at least, to a depletion of endogenous calcium stores in islet cells.

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Arterial stiffness in the Zucker diabetic fatty rat: role of glycemia, insulin resistance, blood pressure, leptin, and sympathovagal activity
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Background and Aims: Arterial stiffness is common in patients with type 2 diabetes, and has been demonstrated to be linked with higher blood pressure, glycemia, insulin level possibly through sympathetic activation, and recently leptin.
Materials and Methods: Different stages of the disease, from the pre-diabetic insulin-resistant stage (Week 6) to the newly diabetic stage (Week 12), and finally to the late insulin-deficient diabetic stage (Week 24), may be considered in the Zucker Diabetic Fatty (ZDF) rat, a model of type 2 diabetes with leptin resistance. Two cannulas, one in the right carotid artery and one in the left femoral artery were implanted after anesthesia in three series of 6–8 ZDF (ZDF/Gmi-*fa/fa*) rats and age-matched lean controls (*?/fa*). Two days after catheterization, pulse pressure waves were simultaneously displayed from conscious rats on a data acquisition system (PowerLab). After blood pressure recording, the rats were euthanazised and the distance between the tips of the 2 catheters measured. Pulse wave velocity (PWV), a marker of arterial stiffness, was calculated by dividing the propagation distance by propagation time. Spectral analysis of the variations in pulse interval (high frequency: PI-HF, representing vagal activity) and systolic blood pressure (low frequency: SBP-LF) was performed.
Results: PWV and blood pressure significantly increased with age (Anova, $p<0.05$ and $p<0.001$, respectively) in both ZDF and control rats, but no difference was observed between the two strains. PI-HF and SBP-LF differed between the two strains ($p<0.05$ for both), when higher PI-HF and lower SBP-LF in the ZDF rats than in the controls. When taking together all ZDF and control rats, PWV correlated positively with blood pressure ($p<0.001$), age ($p<0.01$), and body weight ($p<0.01$), and negatively with PI-HF ($p<0.01$).
Conclusion: The results suggest that, in this model of type 2 diabetes, insulin resistance and hyperglycemia do not seem to be associated with higher arterial stiffness, nor higher blood pressure. Besides leptin resistance, vagal overactivity in the ZDF rats may be protective against arterial rigidity.
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Degenerative neuronal loss in DRG's in long-term type 1 diabetic BB/Wor-rats
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Background and Aims: We have previously reported loss of myelinated and unmyelinated fibers in sural nerves in long-term type 1 diabetic BB/Wor-rats. Here we examined subacute (4 months) and chronic (10 months) spontaneously diabetic BB/Wor-rats with respect to sensory nerve functions, DRG morphometry, pro- and anti-apoptotic proteins and the expression of neurotrophic factors and their receptors.
Results: Sensory nerve conduction velocity was decreased progressively to 78% ($p<0.001$) at 10 mo. Hyperalgesia increased up to 6 mo ($p<0.001$) and returned to normal at 10 mo. The number of DRG neurons decreased significantly to 73% of normal ($p<0.01$) after 10 mo of diabetes. This was almost entirely accounted for a 45% ($p<0.005$) loss of SP neurons and a 48% ($p<0.005$) loss of CGRP neurons. Pro-apoptotic active caspase 3 and Bax expressions in DRG's were increased at both 4 ($p<0.05$, $p<0.005$, respectively) and 10 mo ($p<0.05$, $p<0.01$, respectively). NGF-p75R expression was not altered at 4 mo but increased at 10 mo ($p<0.05$). Anti-apoptotic Bcl-xl and HSP27 expressions in DRG's were increased at 4 mo (both $p<0.05$), but returned to normal at 10 mo. NGF contents in sciatic nerves and expressions of TrkA, IR and IGF-1R in DRG's were significantly decreased at 10 mo. Qualitative morphologic examinations of DRG's revealed no structural evidence of apoptosis, instead neurons demonstrated progressive degenerative changes from small to large vacuolization, cytoplasmic disintegration and the formation of nodules of Nageotte.
Conclusion: The present data show that the presence of diabetes-induced apoptotic stress is counteracted by survival elements. We conclude that DRG neuronal loss is due to hydropic degeneration induced by withdrawal of neurotrophic support.
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Moderate hyperglycaemia does not affect mortality after myocardial infarction in mice

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Background and Aims: Diabetes increases the risk of development of heart failure after myocardial infarction (MI). Former studies investigated usually high glucose levels, the chronic effects of moderately elevated blood glucose on cardiac muscle are unknown. Therefore, we have undertaken a study to assess the consequences of long-term moderate hyperglycemia on survival and left ventricular remodeling following experimental MI in mice.
Materials and Methods: Moderate hyperglycaemia (MH) 200–400 mg/dL, mean: 262,36, SD 97,7 was produced in 37 male C57Bl6/J mice by 5 ip injections of streptozotocin (40 mg/kg); 37 age, sex and strain matched mice served as controls. 8 weeks later mice were subjected to left coronary artery ligation or sham operation. Early infarct size was measured in 20 animals using Evans Blue and TTC 5 hours after MI. Other mice were housed for eight weeks, then their hearts were fixed in situ. Morphometric measurements of LV were performed. Azan blue staining presented the extent of fibrosis and TUNEL-FITC staining revealed frequency of apoptosis in the failing myocardium.
Results: Perfusion with Evans blue revealed comparable area at risk (AAR) in both control and MH mice (37.6%± 12.2% vs 36.4%± 12.2% respectively). Additional TTC staining presented very similar infarct/AAR ratio in both groups (72.4%± 11.9% control vs 70.5%± 16.9% DM). MH mice presented similar mortality during eight weeks as the controls (36.4% vs 37.5%).
Conclusion: Based on our study and previous findings we suggest that prolonged moderate hyperglyceamia, unlike the severe one, does not adversely affect survival after the myocardial infarction in mice.
The unfavorable effect of controlled DM in clinical setting may also be due to increased rate of vascular complications or other elements of metabolic syndrome (hyperinsulinemia or inflammatory response).